OPEN ACCESS

ORIGINAL ARTICLE

Phytochemical screening, median lethal dose and effects of *Cassia abbreviata* Oliv. crude extracts and fractions on oral glucose tolerance in non-diabetic male Wistar rats

Emmanuel Mwila Musenge,[1]* Lavina Prashar,[1] Christian Chinyere Ezeala,[2] Fastone Mathews Goma[1]

[1]*Department of Physiological Sciences, School of Medicine, University of Zambia, Lusaka, Zambia*
[2]*Department of Biomedical and Laboratory Sciences, College of Health, Agriculture, and Natural Sciences, Africa University, Mutare, Zimbabwe*

ABSTRACT

Background: *Cassia abbreviata* plant extracts are used traditionally to treat various ailments including diabetes mellitus. However, very few animal studies have been conducted to investigate potential hypoglycaemic effects locally. We examined phytochemicals, median lethal dose and effects of *Cassia abbreviata* extracts and fractions on oral glucose tolerance in non-diabetic male Wistar rats.

Methods: Qualitative and Lorke's methods were used to screen for phytochemicals and acute toxicity testing. Rats weighing 160 - 250 g were divided into 4 groups of 3 rats (phase one) and 4 groups of 1 rat (phase two) each for acute toxicity. Main experiments comprised 8 groups (extracts) and 7 groups (fractions) of 6 rats per group. Doses of 762 mgkg-1, 381 mgkg-1, and 190 mgkg-1 for extracts, 381 mg.kg-1 for the fractions, 10 mg.kg-1 for sitagliptin and 10 mL.kg-1 of vehicle were administered. Thereafter, blood glucose levels were assessed using Accu-Chek glucometer.

Results: Leaf methanolic extract and ethyl acetate fraction contained more phytochemicals. Acute toxicity test revealed no mortality in all the groups except for leaf ethanolic and methanolic crude extracts in phase two at dose 5000 mg.kg-1. Root bark ethanolic extract 381 mg.kg-1 showed significant hypoglycaemic effect at 60 min and 180 min while 381 mg.kg-1 n-hexane fraction showed significant hypoglycaemic effect only at 180 min. Sitagliptin 10 mg.kg-1 minimally reduced the blood glucose levels at 60 min.

Conclusion: More phytochemicals were observed in leaf methanolic extract and ethyl acetate fraction. Both extracts and fractions were considered practically non-toxic following acute toxicity testing. Root bark ethanolic extract and n-hexane fraction revealed better hypoglycaemic effects.

***Corresponding author:**
Emmanuel Mwila Musenge,
Department of Physiological Sciences, School of Medicine,
University of Zambia, Lusaka, Zambia,
Email: emmanuel.musenge@unza.zm

Keywords: Phytochemicals, median lethal dose, hypoglycaemia, *Cassia abbreviata*, glucose tolerance

This article is available online at: http://www.mjz.co.zm, http://ajol.info/index.php/mjz, doi: https://doi.org/10.55320/mjz.50.3.427

The Medical Journal of Zambia, ISSN 0047-651X, is published by the Zambia Medical Association

© This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Hypoglycaemic bioactivity observed may be due to various mechanisms.

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease sharing its origin and aggravations from food habits, microbial infections, environment and family genetics[1]. The collective agreement on management of DM is glycaemic control to prevent complications[2]. Although, there are numerous chemical agents available to treat DM, these treatments have adverse effects, and not always satisfactory in maintaining euglycaemia[3]. Sixty percent of the world's population relies on herbal medicine to treat their ailments[4]. Globally, there are over 1000 plant species used for treatment of type 2 DM[5]. *Cassia abbreviata* (*C. abbreviata*) Oliv. also known in English and Bemba as "Sjambok or long pod cassia" and "munsoka-nsoka" respectively *belongs to a family of trees called Fabaceae*[6,7]. *C. abbreviata* grows easily and fast from seedlings and wildings, and withstands drought in winter. It is distributed in drier tropical countries of West Africa, East Africa and Southern Africa, often found in the thickets, deciduous woodland and savannah belts of these regions[6]. The plant is widespread and native to Zambia[7,8].

C. abbreviata flowers, leaves, pods, stems, and roots are used traditionally to treat various ailments including DM. In East Africa, it is used for fever, malaria, stomach troubles, uterine complaints, gonorrhea, syphilis, pneumonia and as purgative [9]. The plant is a dysentery and diarrhoea remedy[7] and DM[10] in South Africa. In Malawi, it is used for snakebites and as charm[11]. In Zimbabwe, the infusion or decoction of the dried bark and roots is mainly used for abdominal pains, stomach-aches, blood pressure, constipation, diarrhoea, bloat, toothache, pneumonia and gonorrhea, laxative and as aphrodisiac for men[6]. The infusion of bark is taken by Bembas in Zambia to treat fever and venereal diseases. Valley Tongas in Zambia drink infusion of chopped roots to treat barrenness in women. Furthermore, Lamba, Lenje, Lilima and Swaka of Zambia use the warmed up infusion of the root for toothache by holding it in the mouth[7] .

A number of phytochemicals are found in different parts of *C. abbreviata*, namely alkaloids, glycosides, flavonoids ,saponins, anthraquinones, phenolics, sterols, tannins, terpenoids and terpens[6,12,13]. A wide range of these phytochemicals in the plant species have demonstrated hypoglycaemic bioactivity effected via different mechanisms[14]. Despite their use, only limited data are available regarding safety and efficacy of most individual phytochemicals in preventing or treating chronic ailments such as DM[15]. The median lethal dose (LD_{50}) is one way to measure the short-term toxicity potential of phytochemicals[16]. Establishing toxicity potential of plants taken already or before being taken improves safety. LD_{50} is usually the first test conducted for every chemical before further toxicity tests are carried out[17]. While animal studies have revealed *C. abbreviata* hypoglycaemic effects especially outside Zambia[18-20] ,very few studies have been conducted to investigate this potential locally. Therefore, we examined phytochemicals, LD_{50}, and effects of *C. abbreviata* crude extracts and fractions on oral glucose tolerance in non-diabetic male Wistar rats.

MATERIALS AND METHODS

Plant material

The leaves and root and stem barks of *C. abbreviata* were collected in July 2020 and August 2022 from the bushes of Kamaila Village at **15**°05'02.1"**S**, **28**°17'59.9"**E** in Chisamba District, Zambia. The plants were growing at an altitude of approximately 1,228 m above sea level. The plant species was identified, authenticated and voucher specimen deposited at the Herbarium section in the Department of Biological Sciences (DOBS), School of Natural Sciences (SNS), University of Zambia (UNZA) with accession number UZL 22418.

Preparation of plant extracts and fractions

The collected fresh and healthy leaves and barks were first carefully washed with tap water to remove debris, rinsed completely with distilled water to remove surface contaminants, and then air-dried in shade for three and six weeks respectively. The plant parts were pulverised and weighed using Sinbo grinder blender-GS-PBJ-01 *and* Nimbus® analytical balance correspondingly to yield 1.13 kg (leaf), 1.22 kg (root), and 1.31 kg (stem) of powder material. The measured powder of leaves and barks were macerated using magnetic stir in ethanol and methanol (50 g/250 mL) and extracted twice on each occasion at 18 - 25°C room temperature for 24 - 48 hours to obtain an infusion. The supernatant was decanted, filtered with Ahlstrom filter paper grade 1 to obtain a filtrate. The infusion was concentrated to dryness under reduced pressure at 40°C using Büchi® Rotavapor® RII evaporator. This yielded 22.4 g leaf ethanolic crude extract (LECE), 9.02 g root bark ethanolic crude extract (RBECE), 13.44 g stem bark ethanolic crude extract (SBECE), 26 g leaf methanolic crude extract (LMCE), 17.62 g root bark methanolic crude extract (RBMCE), and 18.8 g stem bark methanolic crude extract (SBMCE) from 100 g of each plant part.

To obtain the fractions, the clinically significant extract of LECE upon administration to animals, was partitioned using liquid-liquid extraction method[21]. The fractions were dried using anhydrous sodium sulphate and evaporated as already explained to yield 1.38 g petroleum ether (PEF), 1.9 g n-Hexane (n-HF), 4.74 g chloroform (CF), 4.64 g ethyl acetate (EAF), 6.02 g n-Butanol (n-BF) and 5.74 g aqueous (AF) masses. Both crude extracts and fractions were stored in air and water proof containers in a refrigerator at 4°C until required for further analysis.

Phytochemical screening of crude extracts and fractions

The determination of the presence of tannins, polyphenols, saponnins, sterols, flavonoids, Alakaloids, cardiac glycosides, reducing sugars, total carbohydrates, amino acids, protein, and terpenoids was achieved by *qualitative* methods[22-25].

Chemicals

The 50% dextrose and sitagliptin phosphate tablets were purchased from Link Pharmacy, Lusaka, Zambia. Dimethyl Sulfoxide 99.7%, Chloroform 99.0%, Petroleum Ether 40-60°C, Ethyl Acetate 99.5%, Methanol 99.8%, n-Hexane 95.0%, Ethanol 99.7% and n-Butanol were purchased from Kansma Investments Ltd and Chemsol Scientific, Lusaka, Zambia. All the chemicals were of Analytical Research grade.

Experimental animals

The experiments were conducted on health adult male Wistar albino rats, aged eight to twelve weeks and weighing 160 - 250 g. They were obtained from DOBS, SNS, UNZA. The animals were housed in colony cages of 6 rats per cage at ambient temperature and humidity of 25 ± 2ºC and 55 ± 10% respectively. They were housed in a standard 12 h light and 12 h dark cycle environment and fed fresh NAMFEED™ standard pellets (National Milling Corp. Ltd., Lusaka, Zambia) and water ad libitum. The rats were allowed to acclimatize to the laboratory environment for seven days before commencement of the experiments[26]. All procedures satisfied ethical standards of the Institution Animal Care and Use Committee of the UNZA Biomedical Research Ethics Committee and National Health Research Authority, Ref. No. 1396-2020 and Ref No: NHRA00005/15/03/2021 respectively.

Experimental design

Acute toxicity study of crude extracts and fractions

The Lorke's[27] arithmetic method was used to determine LD_{50} and safety of extracts and fractions. The Organisation for Economic Co-operation and Development (OECD) 423[28] guide together with Hodge and Sterner[29] toxicity scale were used to determine toxicity. Animals were fasted overnight for 14 – 16 hours and then randomly allocated to four groups for phase one and two with 12 and 4 rats in each phase respectively. Single oral doses of 10, 100 to 1000 mg.kg-1 (first phase) and 1600, 2900 to 5000 mg.kg-1 (second phase) of extracts and fractions

were administered to animals using 16 g round ball tip curved stainless steel gavage needle. Animals were allowed access to feed and water ad libitum. The animals were monitored closely every 30 minutes for the first 3 hours after administration of the extracts and fractions, then hourly for the next 6 hours for any changes in behavioural, neurological and autonomic profiles, and after 24 and 72 hours to identify lethality. Thus, higher, moderate, and lower doses of 762 mg.kg-1, 381 mg.kg-1, and 190 mg.kg-1 corresponding to $1/5^{th}$, $1/10^{th}$ and $1/20^{th}$ of calculated Ld_{50}^{30} of 3807.8 mg.kg-1 were extrapolated and administered to the animals.

Determination of hypoglycaemic effects of crude extracts and fractions

Oral glucose tolerance test (OGTT) was used to determine hypoglycaemic effects of crude extracts and fractions. Animals were divided into 21 groups and 9 groups (n = 6) for the crude extracts and fractions correspondingly and fasted overnight for 14 – 16 hours. The following day animals were either administered 10 mL.kg-1 of 0.5-1% dimethyl sulfoxide (DMSO) as vehicle, 10 mg.kg-1 sitagliptin as standard drug or 190, 381, and 762 mg.kg-1 leaf and root and stem barks of extracts. A dose of 381 mg.kg-1 of each fraction was administered basing on clinically significant dose of LECE. The extracts and fractions were dissolved in 0.5-1% DMSO. Two g.kg-1 of glucose was administered 30 min after feeding the extracts and fractions to animals. Blood was withdrawn from tail vein at baseline, 30 min, 60 min, 120 min, and 180 min of glucose administration. Fasting blood glucose levels (BGLs) were assessed using Accu-Chek glucometer (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

One-way analysis of variance (ANOVA) followed by Dunnett's post hoc multiple comparison test was performed. Shapiro-Wilk test and Levene's *F*-test were used to test for normality and homogeneity of variance respectively. All tests were performed considering significance level at 5%. The results are expressed as mean ± standard error of the mean. Statistical analysis was performed using IBM SPSS statistics Version 24.0.

Results

Phytochemical screening of Cassia abbreviata crude extracts and fractions

The results of the phytochemical screening of the *C. abbreviata* leaf and root and stem bark extracts and leaf fractions have been depicted in tables 1 and 2 respectively.

Table 1: Phytochemical screening of *Cassia abbreviata* crude extracts

Phytochemical	Ethanol			Methanol		
	Leaf	Root bark	Stem bark	Leaf	Root bark	Stem bark
Saponins	-	+	++	+++	++	++
Phenols	+	++	++	+++	++	++
Reducing sugars	++	++	++	++	++	++
Amino acids	-	-	-	-	-	-
Flavanoids	++	++	+	++	++	++
Total carbohydrates	+	++	++	+	++	++
Proteins	-	-	+	-	-	-
Cardiac glycosides	+	++	++	+	++	++
Sterols	+	++	++	++	++	++
Terpenoids	+	++	++	++	++	++
Alkaloids	++	-	+	+++	-	-
Tannins	++	-	-	+++	-	-
Anthroquinones	-	-	-	+	-	-

Key: +++ High concentration, ++ Moderate concentration, + Low concentration, (-) Aabsent

Table 1 shows that the leaf methanolic crude extract contained more phytochemicals followed by the root bark ethanolic extract while the leaf ethanolic crude extract contained less phytochemicals followed by the stem bark ethanolic and root and stem bark methanolic crude extracts.

Table 2 shows that ethyl acetate fraction contained more phytochemicals followed by aqueous and n-butanol fractions while n-hexane contained less phytochemicals followed by chloroform and petroleum ether.

Acute toxicity study of crude extracts and fractions

The results of the acute toxicity studies of the *C. abbreviata* crude extracts and fractions are shown in tables 3 and 4 respectively.

Table 2: Phytochemical screening of *Cassia abbreviata* ethanolic fractions

Phytochemical	Fraction					
	Aqueous	*n*-Butanol	Ethyl acetate	Chloroform	*n*-Hexane	Petroleum ether
Saponins	++	+	+	-	-	-
Phenols	+ +	-	++	-	-	-
Reducing sugars	+	-	++	-	-	-
Amino acids	-	+	-	-	-	-
Flavanoids	+	+	+	-	-	-
Total carbohydrates	++	+	++	-	-	-
Proteins	-	-	-	-	-	-
Cardiac glycosides	+	+	++	+	-	++
Sterols	-	-	++	+	-	-
Terpenoids	++	+	++	+	+	++
Alkaloids	-	-	-	-	-	-

Key: +++ High concentration, ++ Moderate concentration, + Low concentration, (-) Absent

Table 3: Mortality recorded during oral median lethal dose determination for *Cassia abbreviata* crude extracts

	Ethanol			Methanol		
Dose (mg.kg-1 body weight)	Leaf	Root bark	Stem bark	Leaf	Root bark	Stem bark
***Phase one**						
10	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3
Control	0/3	0/3	0/3	0/3	0/3	0/3
****Phase two**						
1600	0/1	0/1	0/1	0/1	0/1	0/1
2900	0/1	0/1	0/1	0/1	0/1	0/1
5000	1/1	0/1	0/1	1/1	0/1	0/1
Control	0/1	0/1	0/1	0/1	0/1	0/1

Key: *(0/3) 0 = number of death, 3 = number of rats used for the test. **(0/1) or (1/1) 0 or 1= number of death, 1 = number of rats used for the test.

Table 4: Mortality recorded during oral median lethal dose determination for the *Cassia abbreviata* ethanolic fractions

Dose (mg.kg- 1 body weight)	aqueous	*n*-Butanol	Ethyl acetate	Chloroform	*n*-Hexane	Petroleum ether
***Phase one**						
10	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3
Control	0/3	0/3	0/3	0/3	0/3	0/3
****Phase two**						
1600	0/1	0/1	0/1	0/1	0/1	0/1
2900	0/1	0/1	0/1	0/1	0/1	0/1
5000	0/1	0/1	0/1	0/1	0/1	0/1
Control	0/1	0/1	0/1	0/1	0/1	0/1

Key: *(0/3) 0 = number of death, 3 = number of rats used for the test . **(0/1) 0 = number of death, 1 = number of rats used for the test .

Tables 3 and 4 shows that, no mortality was recorded in all the experimental groups in 24 hours, after 72 hours and up to two weeks of follow up after oral administration of crude extracts and fractions except for ethanolic and methanolic crude leaf extracts in phase two at dose 5000 mg.kg-1.

Effects of Cassia abbreviata crude extracts and fractions on oral glucose tolerance in non-diabetic male Wistar rats

The effects of oral administration of *C. abbreviata* crude extracts and fractions on oral glucose tolerance in non-diabetic male Wistar rats are shown in tables 5 and 6 correspondingly.

Table 5: Effects of *Cassia abbreviata* leaf and root and stem bark ethanolic and methanolic crude extracts on oral glucose tolerance in non-diabetic male Wistar rats

Group	Blood glucose level (mmol/L)				
	0 Min	30 Min	60 Min	120 Min	180 Min
Negative control (10 mL.kg-1)	3.82±0.15 (Ref)	3.82±0.16 (Ref)	3.85±0.16 (Ref)	3.72±0.12 (Ref)	3.77±0.12 (Ref)
Positive control (10 mL.kg-1)	3.45±0.30 (*P* = .96)	7.15±0.63 (*P* < .001)	5.25±0.24 (*P* = .009)	5.07±0.24[a] (*P* = .048)	4.92±0.26[b] (*P* = .008)
Sitagliptin (10 mg.kg-1)	3.58±0.14 (*P* = 1.00)	5.37±0.19[b] (*P* = .003)	5.17±0.39[a] (*P* = .02)	5.03±0.53 (*P* = .06)	4.55±0.21 (*P* = .17)
RBECE (190 mg.kg-1)	3.73±0.18 (*P* = 1.00)	5.43±0.12[b] (*P* = .002)	5.25±0.09[b] (*P* = .009)	4.98±0.26 (*P* = .08)	4.63±0.13 (*P* = .09)
RBECE (381 mg.kg-1)	3.30±0.17 (*P* = .66)	5.43±0.38[b] (*P* = .002)	5.25±0.14[b] (*P* = .001)	4.95±0.22 (*P* = .09)	4.75±0.31[b] (*P* = .04)
RBECE (762 mg.kg-1)	3.73±0.182 (*P* = 1.00)	5.50±0.13[b] (*P* = .001)	5.35±0.06[b] (*P* = .004)	5.33±0.25[b] (*P* = .009)	4.85±0.16a (*P* = .02)
RBMCE (190 mg.kg-1)	5.08±0.31[b] (*P* = .002)	6.78±0.18[c] (*P* < .001)	5.88±0.42[c] (*P* < .001)	5.35±0.55[b] (*P* = .008)	5.40±0.38[c] (*P* < .001)
RBMCE (381 mg.kg-1)	4.27±0.22 (*P* = .83)	6.33±0.36[c] (*P* < .001)	6.23±0.38[c] (*P* < .001)	5.33±0.39[b] (*P* = .009)	5.42±0.34[c] (*P* < .001)
RBMCE (762 mg.kg-1)	3.87±0.34 (*P* = 1.00)	5.20±0.21[a] (*P* = .01)	6.15±0.35[c] (*P* < .001)	6.42±0.15[c] (*P* < .001)	4.95±0.21[b] (P = .006)
LECE (190 mg.kg-1)	3.20±0.14 (*P* = .44)	6.07±0.31[c] (*P* < .001)	4.45±0.36 (*P* = .76)	4.07±0.43 (*P* = .99)	4.17±0.17 (*P* = .92)
LECE (381 mg.kg-1)	2.87±0.17[a] (*P* = .045)	5.25±0.18[b] (*P* = .009)	4.90±0.28 (*P* = .11)	4.18±0.40 (*P* = .98)	4.03±0.18 (*P* = .99)
LECE (762 mg.kg-1)	3.92±0.14 (*P* = 1.00)	6.72±0.45[c] (*P* < .001)	5.77±0.02[c] (*P* < .001)	5.15±0.17[a] (*P* = .03)	5.00±0.17[b] (*P* = .003)
LMCE (190 mg.kg-1)	4.05±0.19 (*P* = 1.00)	6.47±0.09[c] (*P* < .001)	5.40±0.14[b] (*P* =.003)	5.22±0.13[a] (*P* = .02)	4.88±0.11[a] (*P* = .01)
LMCE (381 mg.kg-1)	3.70±0.12 (*P* = 1.00)	6.20±0.23[c] (*P* < .001)	4.35±0.35 (*P* = .92)	4.65±0.24 (*P* = .36)	4.52±0.15 (*P* = .21)
LMCE (762 mg.kg-1)	4.20±0.23 (*P* = .94)	6.48±0.27[c] (*P* < .001)	5.80±0.30[b] (*P* < .001)	4.28±0.07 (*P* = .92)	4.83±0.09[a] (*P* = .02)
SBECE (190 mg.kg-1)	4.42±0.19 (*P* = .47)	6.95±0.33[c] (*P* < .001)	6.97±0.32[c] (*P* < .001)	5.60±0.18[c] (*P* = .001)	5.53±0.19[c] (*P* < .001)
SBECE (381 mg.kg-1)	3.47±0.31 (*P* = .97)	5.77±0.17[c] (*P* < .001)	6.65±0.28[c] (*P* < .001)	6.05±0.46[c] (*P* < .001)	5.42±0.30[c] (*P* < .001)
SBECE (762 mg.kg-1)	3.62±0.270 (*P* = 1.00)	4.30±0.103 (*P* = .94)	5.87±0.148[c] (*P* < .001)	5.47±0.375[b] (*P* = .003)	4.44±0.156 (*P* = .34)
SBMCE (190 mg.kg-1)	3.93±0.203 (*P* = 1.00)	6.45±0.367[c] (*P* < .001)	5.18±0.380[a] (*P* = .02)	4.92±0.427 (*P* = .11)	4.78±0.363[a] (*P* = .03)
SBMCE (381 mg.kg-1)	4.23±0.255 (*P* = .89)	7.08±0.239[c] (*P* < .001)	6.52±0.250[c] (*P* < .001)	5.80±0.177[c] (*P* < .001)	5.42±0.114[c] (*P* < .001)
SBMCE (762 mg.kg-1)	4.75±0.402[a] (*P* = .05)	6.40±0.266[c] (*P* < .001)	5.88±0.283[c] (*P* <.001)	5.82±0.316[c] (*P* < .001)	5.47±0.259[c] (*P* < .001)

The values are expressed as mean ± SEM (n=6). *P* value: [a]≤ .05, [b]< ,01, [c]<.001 as compared with negative control group using one-way between groups ANOVA followed by Dunnett's post hoc multiple comparison test.

Table 5 shows that 381 mg.kg-1 RBECE revealed significant hypoglycaemic effect at 60 min (P = .001) and 180 min (P = .04) followed by 190 mg.kg-1 SBMCE and 762 mg.kg-1 LMCE which showed significant hypoglycaemic effect at 60 min (P = .02 and P < .001) and 180 min (P = .03 and P = .02) respectively. 10 mg.kg-1 sitagliptin only showed significant hypoglycaemic effect at 60 min (P = .02). However, 190 mg.k-1 SBECE showed least significant hypoglyacemic effects at 120 min (P = .001) and 180 min (P < .001) followed by 762 mg.kg-1 SBMCE at 60 min (P < .001), 120 min (P < .001) and 180 min (P < .001), 381 mg.kg-1 SBMCE at 60 min (P < .001), 120 min (P < .001) and 180 min (P < .001), 381 mg.kg-1 RBMCE at 60 min (P < .001), 120 min (P = .001), and 180 min (P < .001), and 381 mg.kg-1 SBECE at 120 min (P < .001) and 180 min (P < .001).

Table 6 shows that n-HF 381 mg.kg-1 revealed significant hypoglycaemic effect only at 180 min (P = .05) followed by AF 381 mg.kg-1 which showed significant hypoglycaemic effects only at 120 min (P = .03).

Table 6: Effects of *Cassia abbreviata* ethanolic leaf fractions on oral glucose tolerance in non-diabetic male Wistar rats

Group	Blood glucose level (mmol/L)				
	0 Min	30 Min	60 Min	120 Min	180 Min
NC (10 mL.kg-1)	3.82±0.160 (Ref)	3.85±0.157 (Ref)	3.72±0.117 (Ref)	3.77±0.115 (Ref)	3.78±0.126 (Ref)
PC (10 mL.kg-1)	3.45±0.299 (P = .93)	7.15±0.625[c] (P < .001)	5.25±0.240 (P = .19)	5.07±0.240 (P = .17)	4.92±0.263 (P = .07)
Sit (1 0 mg.kg-1)	3.58±0.142 (P = .99)	5.37±0.186 (P = .06)	5.17±0.399 (P = .24)	5.03±0.534 (P = .19)	4.55±0.214 (P = .36)
PEF (381 mg.kg-1)	3.67±0.263 (P = 1.00)	4.30±0.188 (P = .96)	5.22±0.234 (P = .21)	4.17±0.388 (P = .98)	4.09±0.301 (P = .98)
n-HF (381 mg.kg-1)	4.14±0.061 (P = .96)	5.15±0.099 (P = .14)	6.09±0.185[a] (P = .01)	5.29±0.151 (P = .08)	4.97±0.048[a] (P = .05)
CF (381 mg.kg-1)	3.58±0.549 (P = .99)	5.63±0.52[a] (P = .02)	5.98±1.192[a] (P = .02)	4.74±0.802 (P = .444)	4.51±0.592 (P = .42)
EAF (381 mg.kg-1)	3.08±0.315 (P = .36)	4.65±0.691 (P = .61)	4.03±0.485 (P = .99)	3.52±0.486 (P = .99)	3.54±0.419 (P = .99)
n-BF (381 mg.kg-1)	3.10±0.372 (P = .38)	4.26±0.365 (P = .97)	4.45±0.540 (P = .88)	4.11±0.351 (P = .99)	3.92±0.266 (P = 1.00)
AF (381 mg.kg-1)	4.38±0.095 (P = .64)	5.45±0.198[a] (P = .04)	6.38±0.159[b] (P = .004)	5.54±0.157[a] (P = .03)	4.87±0.137 (P = .09)

The values are expressed as mean ± SEM (n=6). P value: [a]≤ .05, [b]< ,01, [c]<.001 as compared with negative control group using one-way between groups ANOVA followed by Dunnett's post hoc multiple comparison test.

DISCUSSION

Diabetes mellitus is a major public health problem that is approaching epidemic proportions worldwide[4]. Oral and parenteral hypoglycaemic agents are the mainstay of treatment of DM and are effective in hyperglycaemic control. However, they have important side-effects and fail to significantly alter the course of DM complications[31]. Medicinal plants used in various folklore medicine are known to play a significant role in the management of DM especially in developing countries where primary care facilities are limited[30]. Evaluation of plant products for treatment of DM is becoming profitable owing to the presence of several bioactive constituents with therapeutic potential[32]. Although *C. abbreviata* has been used in African forklore medicine for the treatment of DM, limited pharmacological findings justify its use as an antidiabetic medicinal plant[33]. Therefore, we investigated the phytochemicals, LD_{50}, and the effects of *C. abbreviata* crude extracts and fractions on oral glucose tolerance in non-diabetic male Wistar rats.

This study showed that *C. abbreviata* LMCE contained higher concentration of saponins, phenols, alkaloids, and tannins followed by RBECE while LECE contained lowest amount of phytochemicals followed by SBECE and SBMCE and RBMCE. Additionally, EAF contained more phenols, reducing sugars, total carbohydrates, cardiac glycosides, sterols, and terpenoids followed by AF and n-BF while n-HF contained less phytochemicals followed by CF and PEF. Similarly, higher amounts of phenols were present in LMCE, followed by leaf chloroform crude Extract while lower concentration was observed in SBMCE[19]. Additionally, tannins, reducing sugars, alkaloids, and sterols were observed in RBECE, anthroquinone glycosides, sterols, and saponins in LECE, tannins, reducing sugars, flavonoids, sterols, phenolics, and proteins in SBECE while tannins, reducing sugars, flavonoids, and saponins were observed in leaf aqueous crude extract (LACE)[12]. In another study, SBMCE contained alkaloids, tannins, flavonoids, saponins, and terpenoids, SBECE contained alkaloids, tannins, flavonoids, saponins, and terpenoids, hot stem bark aqueous crude extract (SBACE) contained alkaloids, sterols, tannins, flavonoids, saponins, and terpenoids, and cold SBACE contained alkaloids, sterols, tannins, flavonoids, saponins, and terpenoids[13]. Further, flavonoids, phenols, tannins, saponins and alkaloids were reported in SBACE and SBMCE[14] while alkaloids, tannins, flavonoids, saponins, terpenoids, sterols, and phenols were observed in SBACE and SBECE[35].

The phenols might be present in higher concentrations in ethanolic and methanolic extracts but not in aqueous extracts because they are degraded by phenol oxidase in aqueous solvent[36]. Since, alkaloids are more soluble in alcohol and sparingly soluble in water, alkaloids are likely to be absent in aqueous extracts of most plant parts[37]. Other studies have reported absence of alkaloids in the aqueous and ethanolic root bark and stem bark respectively[35]. Considering that tannins are soluble in both water and alcohol, they are likely to be more in ethanolic, methanolic, and aqueous extracts and fractions akin to phenols[12,36]. In agreement to this study, previous studies detected similar phytochemicals with varying concentrations attributed to the differences in polarity of solvents and plant part used[35]. The phytochemical yield mainly increase with increasing solvent polarity index and decreases at very high polarity of the solvent[38]. Primarily, phytochemical composition of plant species is influenced by a variety of environmental factors including the geography, climate, soil type, sun exposure, grazing stress, and seasonal changes[39].

The lethality of *C. abbreviata* was established to assist uncover possible harmful effects following short term administration. The current study recorded no mortality in most of the experimental groups in 24 hours, 72 hours and up to two weeks after oral administration of maximum dose of 5000

mg.kg-1 of crude extracts and fractions. Further, there was no change in body weight, behaviour, and food and water consumption in all the non-mortality groups. Nonetheless, mortality was observed with LECE and LMCE in phase two at dose 5000 mgkg-1 within 24 hours of administration. According to toxicity classes of Hodge and Sterner[29] and Lorke[27], any compound with rat oral dose of 5000 mg.kg-1 or more should be considered as practically non-toxic. Similarly, *C. abbreviata* leaf, stem bark and seed aqueous crude extracts were non-toxic on C2C12 mouse skeletal muscle cells at lethal concentration $(LC)_{50}$ > 5000 µg/mL[40]. In another study, *C. abbreviata* stem bark aqueous crude extract LD_{50} was 500 - 750 mg.kg-1 following intraperitoneal administration in mice[41]. Also, *C. abbreviata* stem bark acetonic crude extract inhibited the activities of baker's yeast α-glucosidase with an inhibitory concentration $(IC)_{50}$ of 0.6 mg.mL-1 resulting in inhibition of over 70%[42]. However, oral preparation of *C. abbreviata* methanolic root and stem bark extracts showed high toxicity to the shrimps with LC_{50} value of < 20µg.mL-1 (2.7µg.mL-1) using the brine shrimp lethality test[43].

Assessment of toxicity of herbal medicines is necessary to evaluate their bioactivity for their safe therapeutic utilisation before administration to animals[44]. While *C. abbreviata* has been used in folklore medicine to treat a number of ailments few toxicity studies have previously been performed especially in vivo. Previous studies have showed that *C. abbreviata* is well tolerated at various dosages. Variations of tolerance could be due to among others, use of different methods to establish the lethality with varying results of LD_{50} and LC_{50}. However, toxicity of individual and specific compounds of *C. abbreviata* is yet to be performed to confirm their safety therapeutic utilisation. Therefore, safety evaluation becomes important because *C. abbreviata* contains many different compounds and adverse effects and toxic dosages are mostly unknown[45].

In the present study, RBECE 381 mg.kg-1 showed statistically significant hypoglycaemic effect at 60 min (P = .001) and 180 min (P = .04) followed by SBMCE 190 mg.kg-1 and LMCE 762 mg.kg-1 at 60 min (P = .02 and P < .001) and 180 min (P = .03 and P = .02) respectively. Regarding the fractions, n-HF 381 mg.kg-1 showed statistically significant hypoglycaemic effect only at 180 min (P = .05) followed by AF 381 mg.kg-1 which showed statistically significant hypoglycaemic effects only at 120 min (P = .03). The SBECE 190 mg.kg-1 at 60 (P < .001), 120 (P < .001), and 180 min (P < .001) and SBMCE 762 mg.kg-1 at 60 (P < .001), 120 (P < .001), and 180 min (P < .001) showed slow reduction in blood glucose levels. After rising at 30 min (P = .003), sitagliptin 10 mg.kg-1 minimally reduced BGLs at 60 min (P = .02). Although the BGLs were reduced in most of the groups after 180 min, better and quicker reduction was observed with LECE 381 mg.kg-1 at 60 min (P = .11), 120 min (P = .98) to 180 min (P = .99) and EAF 381 mg.kg-1 at 60 min (P = .99), 120 min (P = .99) and 180 min (P = .99) but results were not non-statistically significant. Sitagliptin 10 mg.kg-1 seemed not to have immediate effects on BGLs at circulation level. While reasonable reduction was observed at 180 min (P = .17), the results were inconclusive. Across most groups, the highest BGLs occurred at 30 min while the lowest occurred at 180 min. Thus, glucose normalization was observed after 180 min and generally, groups with moderate doses revealed better hypoglycaemic effects.

Previous studies reported comparable hypoglycaemic effects of *C. abbreviata* on 2g.kg-1 glucose load. SBECE 150 and 300 mg.kg-1 showed significant hypoglycaemic effects than were observed in 50 mg.kg-1 SBECE after 180 min in male Sprague-Dawley albino rats[18]. Also, SBACE had significant hypoglycaemic effects at 10 mL.kg-1 than at 5mL.kg-1 in male Wistar albino rats after 180 min[20]. In lower dose group of 5 mL.kg-1 SBACE, the pick for BGLs were maintained up to 180 min. In higher SBACE 10 mL.kg-1 dose, a slow

shooting of BGLs was noticed up to 120 min and there after the level was maintained up to 180 min. Additionally, *C. abbreviata* 100 mg.kg-1 SBMCE significantly reduced BGLs the lowest followed by 50 mgkg-1 SBMCE after 180 min in male Wistar albino rats[20]. These studies revealed that higher doses had significant hypoglycaemic effects on oral glucose load. The BGLs in both lower and higher doses differed significantly when compared to control group. However, 10 mL.kg-1 SBACE and SBMCE revealed non-significant rising GBLs at 60 min[20].

In this study, there could be a possibility that 762 mg.kg-1 SBECE and RBMCE and 381 mg.kg-1 n-BF and EAF BGLs did not peak after 30 min as may have compounds that might inhibit absorption of glucose from the intestine. The maintenance of near normal BGLs might be due to slow absorption of glucose. LECE 190 and 381 mg.kg-1, LMCE 381 mg.kg-1, n-BF and EAF 381 mg.kg-1 had lowered BGLs at 60 min through to 180 min may be due to compounds that enhance insulin activity and increase glucose uptake. RBMCE 190 mg.kg-1 and SBMCE 762 mg.kg-1 had sustained maintenance of BGLs at 60 min through to 180 min probably due to some compounds not having any effect on glucose clearance from circulation. Besides, RBMCE 762 mg.kg-1, SBECE 381 and 762 mg.kg-1, and AF and n-BF 381 mg.kg-1 reduced BGLs after 30 min but sustained maintenance was noticed at 60 and 120 min. This may indicate that the extracts and fractions do not have any effect on glucose clearance from the circulation[19]. Further, the clinically significant hypoglycaemic effects of 381 mg.kg-1 EAF and LECE may be due to the presence of more than one antihyperglycaemic principle and their synergic properties. Also, the negative control (NC) group showed lowest blood glucose levels which remained the same throughout the 180 min experiment. Similar observation were made in SBMCE[30] and LMCE and control group BGLs were maintained up to 180 min[20]. The current study, revealed that doses used had some effect on glucose load that was administered. Some groups exhibited better hypoglycaemic effect than sitagliptin-treated group. Sitagliptin increases glucose-dependent insulin production from pancreatic islet β-cells and decreases hepatic glucose overproduction from pancreatic islet α-cells by prolonging the action of GLP-1 and GIP via inhibition of dipeptidyl-peptidase (DPP) IV enzyme[46].

Although the exact mechanism of action of *C. abbreviata* is unknown, various mechanisms have been proposed. Firstly, it may enhance glucagon-like peptide 1 and 2 (GLP-1 and -2) from enteroendocine L cells and glucose-dependent insulinotropic polypeptide (GIP) from enteroendocrine K cells. GLP-1 and GIP stimulates glucose-dependent insulin release from pancreatic islet β-cells, on ingestion of nutrients[46,47]. Additionally, GLP-1 stimulates glucose disposal[47] and inhibit inappropriate postprandial glucagon release[48]. GLP-2 increases insulin sensitivity and suppresses basal hepatic glucose production[49] and regulate intestinal hexose transporters which enhances glucose absorption[50]. Secondly, *C. abbreviata* might increase synthesis of insulin with consequent increase in plasma levels[20,30]. Finally, it could increase glucose uptake by adipose, liver and muscle tissues[18]. The increased binding of insulin to insulin receptor enhance the uptake of glucose by extract and fractions as demonstrated in rat hemidiaphragm experiment[30,40].

LIMITATIONS

The present study did not investigate the pure compounds in the extracts nor fractions of *C. abbreviata*. Therefore, hypoglycaemic effects observed may differ if pure compounds were used. Although this study confirms that *C. abbreviata* is practically safe, the acute toxicity that was conducted may not reveal the long term effects and toxicity to important organs of animals such as kidney, liver, heart and spleen. Equally, this study may not reveal the long term effectiveness of the extracts and fractions. In any case, the study revealed safe doses and hypoglycaemic effects of extracts and fractions on acute hyperglycaemia.

CONCLUSION

This study revealed that LMCE and EAF contained higher concentration of various phytochemicals while the LECE and n-HF contained the lower concentration of phytochemicals. Acute toxicity results showed that, mortality was recorded only in 5000 mg.kg-1 LECE and LMCE groups in phase two of LD_{50}. It may be concluded that most of the extracts and all fractions were practically safe. RBECE 381 mg.kg-1 revealed significant hypoglycaemic effect at 60 and 180 min followed by SBMCE 190 mg.kg-1 and LMCE 762 mg.kg-1 at 60 and 180 min respectively. Sitagliptin 10 mg.kg-1 only showed significant hypoglycaemic effect at 60 min. SBECE 190 mg.k-1 showed least significant hypoglyacemic effects at 120 min and 180 min followed by SBMCE 762 mg.kg-1 and SBMCE 381 mg.kg-1 at 60 min, 120 min and 180 min respectively. n-HF 381 mg.kg-1 revealed significant hypoglycaemic effect only at 180 min followed by AF 381 mg.kg-1 only at 120 min. However, 381 mg.kg-1 LECE and EAF revealed clinically significant hypoglycaemic effects. Although, significant concentration of phytochemicals were observed in LMCE and EAF, RBECE with moderate amounts of phytochemicals significantly reduced BGLs after 180 min. The hypoglycaemic effects observed in different groups could be due to the presence of bioactive compounds in *C. abbreviata.* The reduction in BGLs could be due to delayed absorption of glucose, increased insulin release or enhanced glucose uptake by muscles. Attempts to purify EAF and investigating regeneration of islet β-cells and responsible transcription factors are underway.

***What is already known on this topic*:**

- *C. abbreviata* contains various phytochemicals such as phenols, flavonoids, alkaloids, tannins, saponnins among others
- Phytochemicals in *C. abbreviata* have demonstrated hypoglycaemic effects both in vitro and in vivo
- Different dosages in different studies have been used to effect hypoglycaemia

***What this study adds*:**

- Investigation of different parts of *C. abbreviata* in one study
- Fractionation of clinically significant leaf ethanolic crude extract into six fractions of petroleum ether, n-hexane, chloroform, ethyl acetate, n-butanol, and aqueous
- Investigation of *C. abbreviata* plant species due to paucity of data on its hypoglycaemic effects in locally

Acknowledgements

The authors would like to appreciate the Departments of Physiological Sciences and Chemistry in the Schools of Medicine and Natural Sciences of UNZA respectively for granting us access to the facilities. Special gratitude goes to the staff in the animal house and laboratories of these departments for the support and guidance. Finally, our gratefulness go to the Training and Development Office of UNZA for the award of the special research fellowship and support.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

EMM conceived the study. EMM, LP, CCE and FMG designed the study. EMM conducted the study and analysed the data. LP, CCE and FMG supervised the whole study process, including the writing of the manuscript. All the authors wrote and approved the final version of the manuscript.

Funding

There was no specific funding for this article.

REFERENCES

1. Radha B, Muniraj G, Rasu R. The Making of Pancreatic β Cells: Advances and Apprehensions. *Int J Pharmacol Phytochem Ethnomedicine*. 2016;5:34-51. doi:10.18052/www.scipress.com/IJPPE.5.34
2. Skrzypek K, Groot Nibbelink M, Van Lente J, et al. Pancreatic islet macroencapsulation using microwell porous membranes. *Sci Rep*. 2017;7(1):9186. doi:10.1038/s41598-017-09647-7
3. Yagihashi S, Inaba W, Mizukami H. Dynamic pathology of islet endocrine cells in type 2 diabetes: β-Cell growth, death, regeneration and their clinical implications. *J Diabetes Investig*. 2016;7(2):155-165. doi:10.1111/jdi.12424
4. WHO. Diabetes fact sheet No312. Published online 2015.
5. Rahman IU, Bashir M, Salman M, Idrees M, Khan MI. Bitter Melon (Momordica Charantia) Reduces Serum Sialic Acid in Type2 Diabetics: Evidence to Delay the Process of Atherosclerosis. *Chin Med*. 2011;02(04):125-129. doi:10.4236/cm.2011.24021
6. Viol DI. Screening of traditional medicinal plants from Zimbabwe for phytochemistry, antioxidant, antimicrobial, antiviral and toxicological activities. Published online August 2, 2013. Accessed December 8, 2023. https://zdhr.uz.ac.zw/xmlui/handle/123456789/1091
7. Fanshawe DB. *Useful Trees of Zambia for the Agriculturist. Compiled by D. B. Fanshawe. With Line Drawings by Mrs. Alvin Watkins et Al*. Ministry of Lands and Natural Resources; 1972.
8. Chisumpa, S.M,. Diversity in forest species. An overview of forest resources. In proceedings of the first National Plant Genetic Resources. In: ; 1990.
9. Kokwaro, J.O. *Medicinal Plants of East Africa.* East African Literature Bureau; 1976.
10. Chauke M, Shai L, Mogale M, Tshisikhawe M, Mokgotho M. Medicinal plant use of villagers in the Mopani district, Limpopo province, South Africa. *Afr J Tradit Complement Altern Med*. 2015;12(3):9-26. doi:10.4314/ajtcam.v12i3.2
11. Williamson, J.,. *Useful Plants of Malawi. Revised and Extended Edition.* Montfort Press.; 1975.
12. Kabuka R, Mudenda S, Kampamba M, Chulu M, Chimombe T, Hikaambo CN. Phytochemical Analysis of Leaf, Stem Bark, and Root Extracts of Cassia abbreviata Grown in Zambia. *Pharmacol Amp Pharm*. 2022;13(05):119-128. doi:10.4236/pp.2022.135009
13. Chitoti J, Kaonga P, Prasha L. Anti-Diarrhoeal Properties of Stem Bark of Cassia Abbreviataagainst Castor Oil-Induced Diarrhoea in Swiss Albino Mice. *Med J Zambia*. 2021;47(3):252-260. doi:10.55320/mjz.47.4.117
14. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*. 2012;2(4):320-330. doi:10.1016/S2221-1691(12)60032-X
15. Bode AM, Dong Z. Toxic phytochemicals and their potential risks for human cancer. *Cancer Prev Res Phila Pa*. 2015;8(1):1-8. doi:10.1158/1940-6207.CAPR-14-0160
16. Saganuwan S. Toxicity studies of drugs and chemicals in animals: an overview. *Bulg J Vet Med*. 2017;20(4):291-318. doi:10.15547/bjvm.983
17. Nusrat K, Maheshwari DG. An overview on-toxicity testing methods. 2016;8(2):3834-3849.
18. Bati K, Kwape TE, Chaturvedi P. Anti-Diabetic Effects of an Ethanol Extract of Cassia Abbreviata Stem Bark on Diabetic Rats and Possible Mechanism of Its Action: - Anti-diabetic Properties of Cassia abbreviata. *J*

Pharmacopuncture. 2017;20(1):45-51. doi:10.3831/KPI.2017.20.008

19. Chaturvedi Padmaja, Monubung Katlego and Ndubiwa Tshephang Ndubiwa. Anti-diabetic and free radical scavenging potentials of cassia abbreviata. *European Journal of Biomedical and Pharmaceutical sciences,*. 2016;3(12):119-124.
20. Chaturvedi Padmaja, Namposya Daka Rabecca, and Tedora Habtai Aibu. Cassia abbreviata extracts prevent the ensuing pre-diabetes in albino rats subjected to sucrose load. *J Appl Zool Res*. 2016;27(1):33-39.
21. Chattopadhyay RR. Possible mechanism of antihyperglycemic effect of Gymnema sylvestre leaf extract, part I. *Gen Pharmacol*. 1998;31(3):495-496. doi:10.1016/s0306-3623(97)00450-3
22. Alamzeb M, Khan MR, Ali S, Shah SQ, Rashid MU. Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*. *Bangladesh J Pharmacol*. 2013;8(2):107-109. doi:10.3329/bjp.v8i2.13551
23. Godghate A, Sawant R, Sutar A. Phytochemical analysis of ethanolic extract of roots of Carrisa carandus Linn. *Rasayan J Chem*. 2012;5(4):456-459.
24. Kumar J, Chezhian A, Senthilraja P. Computational selections of terpenes present in the plant Calotropis gigantea as mosquito larvicide's by blocking the sterol carrying protein, AeSCP-2. *Bangladesh J Pharmacol*. 2012;7(1)× 1-5:1-5.
25. Tyagi T, Agarwal M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms. *J Pharmacogn Phytochem*. 2017;6(1):195-206.
26. Jere E, Ezeala CC, Prashar L. Phytochemical Studies and Blood Glucose Lowering Effect of Zambian Cultivars of Piliostigma thonningii in Alloxan - Induced Diabetic Rats. *Med J Zambia*. 2021;48(2):70-77. doi:10.55320/mjz.48.2.28
27. Lorke, D. A new approach to tropical acute toxicity testing. *Arch Toxicol*. 1983;53:275-287.
28. OECD. Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Published online 2000. https://doi.org/10.1787/9789264078376-en
29. Hodge, A. and Sterner, B. Toxicity Classes. In: Canadian Center for Occupational Health and Safety. Published online 2005. http://www.ccohs.ca/oshanswers/chemicals/id50.htm
30. Einstein JW, Mohd Rais M, Mohd MA. Comparative Evaluation of the Antidiabetic Effects of Different Parts of *Cassia fistula* Linn, a Southeast Asian Plant. *J Chem*. 2013;2013:1-10. doi:10.1155/2013/714063
31. Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol*. 2004;92(1):1-21. doi:10.1016/j.jep.2003.12.031
32. Bindu Jacob null, Narendhirakannan R T null. Role of medicinal plants in the management of diabetes mellitus: a review. *3 Biotech*. 2019;9(1):4. doi:10.1007/s13205-018-1528-0
33. Usai R, Majoni S, Rwere F. Natural products for the treatment and management of diabetes mellitus in Zimbabwe-a review. *Front Pharmacol*. 2022;13:980819. doi:10.3389/fphar.2022.980819
34. Msiska T, Mwakikunga A, Tembo D, Musopole A, Lampiao F. The in Vivo Effects of Cassia Abbreviata Oliv., on Sperm Parameters And Testicular Histology of The Male Wistar Rat. *Curr Tradit Med*. 2021;7(3):447-456. doi:10.2174/2215083806999200711162520
35. Hikaambo C, Kaacha L, Mudenda S, et al. Phytochemical Analysis and Antibacterial

Activity of Azadirachta indica Leaf Extracts against Escherichia coli. *Pharmacol Pharm*. 2022;13:1-10. doi:10.4236/pp.2022.131001

36. Jobe MC, Ncobela CN, Kunene NW, Opoku AR. Effects of Cassia abbreviata extract and stocking density on growth performance, oxidative stress and liver function of indigenous chickens. *Trop Anim Health Prod*. 2019;51(8):2567-2574. doi:10.1007/s11250-019-01979-y
37. Aristide T, Sylvin O, Mohamed BB, Adama K, Innocent PG. Phytochemical analysis and ovicidal activity of Cassia sieberiana, Guiera senegalensis and Excoecaria grahamii extracts. *Afr J Pharm Pharmacol*. 2017;11(44):554-560. doi:10.5897/AJPP2017.4837
38. Wakeel A, Jan SA, Ullah I, Shinwari ZK, Xu M. Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. *PeerJ*. 2019;7:e7857. doi:10.7717/peerj.7857
39. Kumar S, Yadav M, Yadav A, Yadav JP. Impact of spatial and climatic conditions on phytochemical diversity and in vitro antioxidant activity of Indian Aloe vera (L.) Burm.f. *South Afr J Bot*. 2017;111:50-59. doi:10.1016/j.sajb.2017.03.012
40. Kamga-Simo FDY, Kamatou GP, Ssemakalu C, Shai LJ. Cassia Abbreviata Enhances Glucose Uptake and Glucose Transporter 4 Translocation in C2C12 Mouse Skeletal Muscle Cells. *J Evid-Based Integr Med*. 2021;26:2515690X2110063. doi:10.1177/2515690X211006333
41. Parry O, Matambo C. Some pharmacological actions of aloe extracts and Cassia abbreviata on rats and mice. *Cent Afr J Med*. 1992;38(10):409-414.
42. Shai LJ, Masoko P, Mokgotho MP, et al. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South Afr J Bot*. 2010;76(3):465-470. doi:10.1016/j.sajb.2010.03.002
43. Moshi M, Van Den Beukel C, Hamza O, et al. Brine Shrimp Toxicity Evaluation Of Some Tanzanian Plants Used Traditionally For The Treatment Of Fungal Infections. *Afr J Tradit Complement Altern Med*. 2008;4(2):219-225. doi:10.4314/ajtcam.v4i2.31211
44. Ahmed S, Qureshi B, Hasan M, Ahmed S, Azhar I. Toxicity assessment of Mucuna pruriens Linn seeds. *Int Res J Pharm*. 2011;2:133-135.
45. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*. 2014;4. doi:10.3389/fphar.2013.00177
46. Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: Similarities and differences. *J Diabetes Investig*. 2010;1(1-2):8-23. doi:10.1111/j.2040-1124.2010.00022.x
47. Lee YS, Jun HS. Anti-diabetic actions of glucagon-like peptide-1 on pancreatic beta-cells. *Metabolism*. 2014;63(1):9-19. doi:10.1016/j.metabol.2013.09.010
48. Koliaki C, Doupis J. Incretin-based therapy: a powerful and promising weapon in the treatment of type 2 diabetes mellitus. *Diabetes Ther*. 2011;2(2):101-121. doi:10.1007/s13300-011-0002-3
49. Shi X, Zhou F, Li X, et al. Central GLP-2 enhances hepatic insulin sensitivity via activating PI3K signaling in POMC neurons. *Cell Metab*. 2013;18(1):86-98. doi:10.1016/j.cmet.2013.06.014
50. Ejarque M, Sabadell-Basallote J, Beiroa D, et al. Adipose tissue is a key organ for the beneficial effects of GLP-2 metabolic function. *Br J Pharmacol*. 2021;178(10):2131-2145. doi:10.1111/bph.15278