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Hypoglycemic activities and biochemical parameters modulation of herbal formulations of Allium cepa L. in alloxanized diabetic rats

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The aim of this work was to formulate and evaluate the herbal tablets of *Allium cepa* L. for *in vitro* tablet properties, hypoglycemic effects and for their effects on some biochemical parameters in diabetic rats. Aqueous extract of *A. cepa* L. was obtained by cold maceration and freeze dried. *A. cepa* L. extract compatibility with some tablet excipients was assessed using Fourier transform infrared spectroscopy. *A. cepa L.* tablets consisted of the extract, gelatin, polyvinyl pyrrolidone (PVP), carboxymethyl cellulose (CMC), Primogel®, Ac-Di-Sol®, Avicel® PH102, anhydrous lactose, talc and stearic acid. Tablets were prepared according to standard physical properties using wet granulation method. The tablets were evaluated for antidiabetic properties and for their effects on some biochemical parameters in alloxan induced diabetic rats with glibenclamide and distilled water as positive and negative controls respectively. The extract-excipient compatibility test indicated compatibility of the extract with gelatin, PVP, CMC, Primogel®, Ac-Di-Sol®, Avicel® PH102, and anhydrous lactose. The prepared tablets maintained adequate mechanical integrity and dissolution profiles after storage for six months. There was significant reduction in blood glucose level upon daily administration of the tablets compared to the negative control (p = 0.05). Maximum reduction of blood glucose levels ranging from 20 to 70% were achieved within 21 days of daily administration of the tablets. The plasma levels of liver enzymes such as alanine transaminase, aspartate transaminase and alkaline phosphatase were significantly reduced together with total cholesterol and triglycerides. There were also reduced activities of catalase, glutathione reductase and malondialdehyde.

Key words: *Allium cepa*, hypoglycemic effects, biochemical parameters, alloxan, wet granulation.

INTRODUCTION

Plant products are increasingly being used as medicinal products, nutraceuticals and cosmetics. They are mainly used in the treatment of chronic diseases such as hypertension, arthritis, diabetes mellitus, cancer, cough

remedies, memory loss, etc (Patel et al., 2006). According to the World Health Organization (WHO) (2008), about 80% of the world population use herbal and traditional medicine for their primary health care needs. The upsurge in the use of herbal products can be linked to the global trend of many people seeking to return to nature. These products also include herbal supplements taken to promote health. They are normally sold as herbal tablets, capsules, powders, liquid extracts, and fresh or dried plants. In the United States of America (USA), herbal supplements are classified as dietary supplements by the United States Dietary Supplement, Health, and Education Act (DSHEA) (1994). Part of the provisions of this act is that herbal products can be sold unlike the prescription medicine, without being tested to prove that they are safe and effective. However, this herbal supplements must be prepared according to current good manufacturing practices (UMMC, 2017).

The use of herbal products in treatment of diabetes mellitus is on the increase due to their efficacy and safety (Hakim et al., 2007). Diabetes mellitus commonly called diabetes is a metabolic disease characterized by chronic hyperglycemia due to defective insulin secretion or insulin action or both leading to disorder in carbohydrate, lipid and protein metabolism (Lebovitz, 1994; Akah et al., 2009). Diabetes is a chronic disease and is presently incurable, but treatment is directed toward relief of symptoms such as hyperglycemia, polyuria, glycosuria etc (Milton, 1976). In Allopathic medicine, diabetes is managed by non-pharmacological approaches such as dietary restriction, exercise, surgery, and by pharmacological approaches which include insulin and hypoglycemic agents such as sulphonylureas, biguanides, α-glucosidase inhibitors, thiazolidinedione and many other hypoglycemic agents (Adeneye and Agbaje, 2008; Emeje et al., 2011). Even though a lot of progress has been made in treatment of diabetes, search for newer drugs is still continuing due to the side effects of these medications and their inability to control long term complications of diabetes. Attention has been shifted to medicinal plants and herbal medications with potential antidiabetic properties such as *A. cepa* L. This is a common household vegetable and also used in folk medicine for the treatment of bruises, colic, ear ache, bronchitis, cold, fevers, intestinal parasites, diabetes, high blood pressure, sores and impotence (WHO, 1995). This house hold vegetable can lead to production of cheap and affordable medicines in line with WHO recommendation that traditional medicines should be integrated into the national health care delivery system (Nwanjo, 2005).

A. cepa L. belongs to the family of Liliaceae and has been reported to have strong antidiabetic properties which are attributed to the several of its constituents (Lanzotti, 2006; Kumari et al., 1995; Ogbonna and Ofoefule, 2016). Although diabetes is caused by many factors, most of the orthodox hypoglycemic agents target one pathway of hyperglycemic control. A new approach

to the treatment of diabetes is to target several pathways using one agent or preparation (El-Abhar and Schaalan, 2014). These pathways include but not limited to; stimulation of insulin release, inhibition of gluconeogenesis, reduction of absorption of glucose from intestinal lumen and increase in peripheral glucose utilization (Jyothi et al., 2017). Also, resistance to multi-component preparations as with herbs does not develop quickly (Isimi et al., 2003). Medicinal plants such as *A. cepa* L. can be useful this way. However herbal preparations have the problem of being poor in presentation and unhygienic resulting in low patronage and poor compliance.

This study was aimed at developing oral tablet formulations from the aqueous extract of *Allium cepa L.* containing antidiabetic constituents which may act by several mechanisms to control hyperglycemia. It was also our aim to produce safe, presentable, cheaper and effective antidiabetic tablets that can serve as alternative to orthodox hypoglycemic agents. The availability of the raw materials and ease of propagation of *A. cepa* L. are added advantages which will ensure sustainable supplies during research, clinical trials and subsequent commercialization.

MATERIALS AND METHODS

Plant material

Onion bulbs (*A. cepa* L.) were bought from Ogige market in Nsukka in Enugu State of Nigeria. The identity of the plant was established by Mr. A. O. Ozioko, a plant taxonomist that works with International Centre for Ethnomedicine and Drug Development (InterCEDD) Nsukka. Voucher specimens were deposited at the herbarium unit of Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka with number PCG/UNN/0061.

Animals

Adult male wistar rats (110-150 g) were used. The animals were obtained from the animal house of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria. All the procedures performed in this study that involved the use of animals were done in accordance with ethical standards of the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (Approval Reference Number: FVM-UNN-IACUC-2018-059), which is in compliance with all applicable national and international guidelines.

Chemicals

Alloxan monohydrate and glibenclamide were bought of Sigma chemical Co. USA. Micro crystalline cellulose (Avicel® PH102) was a gift from FMC Corp., USA, and agglomerated lactose was obtained from Meggle, Germany. Chemicals of analytical grades were used in all the experiments where applicable.

Preparation of the extract

The aqueous extract of *A. cepa L.* was prepared by cold maceration

method. A 2000 g quantity of *A. cepa* bulbs were sliced and homogenized by means of a blender (Panasonic, Japan). The pulp was then extracted by soaking it in distilled water for 72 h with occasional agitation and filtered. The filtrate was then freeze dried with Christ alpha 1-2 LD model freeze drier (Sciquib Ltd, Shropshire,U.K.) to obtain the solid extract.

Preparation of the *A. cepa* L. extracts granules

Granules of *A. cepa L.* extract were prepared by using standard wet granulation method (Sravya, 2016). The formulation ingredients were weighed out according to the formula stated in Table 1. Granules equivalent to a batch of 100 tablets were prepared by using the stated binder to prepare a 20% w/v granulating solution which was used to wet mass the weighed amount of the extract, the filler and half of the disintegrant. In preparing batch A_1, into a well cleaned and dried mortar were placed 10 g of the extract, 17 g of the filler (1:1 Avicel[®] + lactose) and half of the maize starch disintegrants (0.75 g). A 0.9 g quantity of gelatin was weighed out and used to prepare 20% w/v mucilage by dispersing the 0.9 g of gelatin in 4.5 ml of boiled distilled water. The gelatin mucilage was added to the ingredients in the mortar and used to wet mass the other ingredients in the mortar by kneading in the mortar for 30 min to form a wet mass. The wet mass was screened through a sieve of 1.7 mm mesh size to give the wet granules. These wet granules were dried in an oven maintained at 50°C for one hour. The dried granules were then screened again through sieve of 1.0 mm mesh size to obtain the dry granules. The dried granules were stored in a well closed dry bottles awaiting compression.

Determination of drug/excipients compatibility

Fourier transform infra-red (FTIR) spectroscopy

The formulated granulates; excipients together with the extract were separately subjected to Fourier FTIR spectroscopy using FTIR spectrophotometer (FTIR-230 (Jasco Co., Japan). The spectral data obtained were analyzed for drug interactions and functional groups.

Evaluation of the granules properties

Bulk and tapped densities

A 10 g weight of each of the extract/excipients blend or granules was weighed out and put in a 50 ml measuring cylinder and allowed to drop on the table from a height of about 10 cm. The volume occupied by the blend/granules was read directly from the measuring cylinder and recorded as the bulk volume. Tapped volume for each batch was obtained by fixing the measuring cylinder containing a known weight of each batch on an automatic tapping machine Stampf volumeter (Karl Klobb, Dreieich, Germany). The tapping by the machine continued until a constant volume was reached and this was noted as the tapped volume. The bulk and tapped densities were the calculated using Equations 1 and 2.

$$\text{Bulk density } (D_b) = \frac{M}{V_b}$$
(1)

$$\text{Tapped density } (D_t) = \frac{M}{V_t}$$
(2)

Where M is the mass of the granules, V_b is the bulk volume and V_t

is the tapped volume in ml.

Hausner quotient and Carr's compressibility index

Hausner's quotient (HQ) and Carr's compressibility index (CI) were calculated using the equations given below.

$$\text{HQ} = \frac{D_t}{D_b}$$
(3)

$$\text{C.I. (\%)} = \frac{D_t - D_b}{D_t} \times 100$$
(4)

True density

The true density (D_T) of each batch was determined by fluid displacement method using non solvent fluid xylene (Armstrong et al., 1989). A 50 ml pycnometer density bottle was used. The weight of the pycnometer was noted. The density bottle was then filled with xylene and the new weight (W_1) was noted. A known weight W (1 g) of each batch was added in respective measurements and the weight of the xylene with density bottle and the sample was noted as (W_2). True density was then calculated from the Equation 5.

$$D_T = \frac{W}{W_1 + W - W_2} \times \text{S.G}$$
(5)

Where; S.G. is the specific gravity of the non-solvent xylene.

Flow rate and angle of repose

The method described by Carstesen and Chan (1977) was used in determination of the flow rate and angle of repose. A plastic funnel was placed in metal ring support clamped on to a retort stand and placed 10 cm above the bench top. The orifice of the funnel was closed temporarily with small sheet of cardboard paper and a 10 g quantity of each granule batch was poured into the funnel. The sheet of paper covering the orifice was removed and a stop watch timer started simultaneously. The granules were allowed to flow freely on to a plane sheet of paper. The time of flow t(s) that is, time taken for the sample to flow from the funnel till all the powder had passed through the orifice of the funnel, height of heap (h) formed and diameter (d) of heap formed were noted. The flow rate (F) and angle of repose (θ) were calculated using the Equations 6 and 8 respectively.

F = Mass (g)/Time of flow (s)
(6)

Tan θ = h/0.5d
(7)

$$\theta = \text{Tan}^{-1} h/0.5d$$
(8)

Particle size analysis

Particle size analysis was done using the sieve method. A known weight of granules or blends was passed through a set of 7 sieves ranging from 1000 – 150 µm arranged in order of decreasing mesh size that is, sieve with highest mesh size on top and the lowest at the bottom and inserted inside the plate of the sieve shaker. A Retsch sieve shaker Type AS 200 (Retsch GMBH and CO. Germany) was used to shake the sieves for 10 min and the quantity

Table 1. Composition of the *A. cepa* L. tablet (mg).

Bch	Extract	Maize starch	Gelatin	PVP	Na CMC	Prim.[®]	Ac.®	Maize starch	Filler	Talc	St. acid	Tot. wt
A$_1$	100	-	9	-	-	-	-	15	170	3	3	300
A$_2$	100	-	9	-	-	12	-	-	173	3	3	300
A$_3$	100	-	9	-	-	-	9	-	176	3	3	300
B$_1$	100	-	-	15	-	-	-	15	164	3	3	300
B$_2$	100	-	-	15	-	12	-	-	167	3	3	300
B$_3$	100	-	-	15	-	-	9	-	170	3	3	300
C$_1$	100	-	-	-	15	-	-	15	164	3	3	300
C$_2$	100	-	-	-	15	12	-	-	167	3	3	300
C$_3$	100	-	-	-	15	-	9	-	170	3	3	300
D$_1$	100	15	-	-	-	-	-	15	164	3	3	300
D$_2$	100	15	-	-	-	12	-	-	167	3	3	300
D$_3$	100	15	-	-	-	-	9	-	170	3	3	300

Bch = batch, filler = Avicel® + lactose [1:1], PVP = polyvinyl pyrrolidone, Prim. = Primogel®), Ac = Ac-Di-Sol®, NaCMC = sodium carboxyl methylcellulose, St. acid = stearic acid, tot. wt = total weight.

of each sample held by each sieve was weighed and recorded. Particle size distribution was calculated as the ratio of the sieve cumulative weight and the total weight of the sample.

Preparation of the A. cepa L. extract tablets

The different batches of the herbal tablets were prepared according to the composition as shown in Table 1. Prior to compression, the granules were then screened through sieve of mesh size 250 µm to separate the fines. The remaining portion of the maize starch was added to the fines and mixed and then lubricated by adding 300 mg respectively of stearic acid and talc and mixed for 3 min. The lubricated fines were then re-mixed with the coarse granules for another 3 min and compressed into tablets. Compression of the granules into tablets was carried out using Manesty F3 No 18L174 (England) single punch machine with 9 mm concave punches. The compaction force was set in such a way as to achieve similar hardness for each batch. A total of twelve batches A$_1$ – D$_3$ were prepared.

Evaluation of the A. cepa herbal tablets properties

The various batches of tablets prepared were evaluated for the following properties.

Weight uniformity test

Twenty tablets were selected randomly from each batch and weighed together and singly with digital balance (Adventurer™, Ohaus, China). The mean weight, standard deviation and coefficient of variation were determined.

Hardness test

Mosanto hardness tester (Manesty model F3, England) was used to measure the hardness of 10 randomly selected tablets from each batch. Each tablet was placed between the jaws of the tester and the tester adjusted to zero. Pressure was added by turning the screw in a clockwise direction until the tablet got broken

diametrically. The value was then recorded. The mean of 10 measurements and standard deviation were then calculated.

Friability test

Roche friabilator (Erweka, Type Tar Nr 50234, Germany) was used to determine the percentage loss in weight of the tablet batches after being subjected to the specified level of agitation in the closed chamber of the friabilator. Twenty tablets randomly selected from each batch were first of all de-dusted and weighed together (W_o) and was put into Roche friabilator and the machine was allowed to operate for four minutes at the rate speed of 25 rotations per minute. The tablets were then dedusted again and reweighed (W) and the percentage loss in weight or the friability (F) was calculated using the Equation 9.

$$F\ (\%) = \frac{W_O - W}{W_O}\ \text{X100}$$

(9)

Disintegration time test

Disintegration time is the time required for a breakdown of a tablet into smaller particles that pass through the 2 mm screen completely (Ofoefule, 2002). The disintegration time test was carried out using Erweka disintegration machine (Erweka, type ZT4 Nr32440, Germany). Distilled water maintained at the temperature of 37.0±1.0°C was used as the disintegration medium. Six randomly selected tablets from each batch were put singly into each tube of the disintegration unit whose lower end was closed by a screen of 2 mm aperture. The tubes were raised up and lowered in a bath containing the disintegration medium steadily until the tablet breaks up and the fragments pass through the mesh of the tube. Tablets are said to be disintegrated if no fragments remain on the screen. The time taken for this to happen was noted. The mean and the standard deviation were calculated.

Content of active ingredient

The tablets were assayed spectrophotometrically using UV-VIS

spectrophotometer (UV-1900, Shimadzu, Japan). A standard Beer's calibration curve was prepared at 289 nm which was the wavelength of maximum absorption following a scan. This was done by preparing several dilutions of the extract in 0.1 N HCl and their absorbance taken. A plot of absorbance versus concentration yielded the standard Beer's plot which was subsequently used for assay of the herbal tablets and also for the dissolution studies.

The content of active ingredient was evaluated for each batch. Twenty tablets were randomly selected and crushed in a mortar. An amount equivalent to the theoretical content of each tablet was weighed out using a sensitive digital balance (Adventurer™, Ohaus SNR 1121253860, China). The weighed powder was put in 100 ml volumetric flask and dissolved in 100 ml distilled water. A 5 ml portion was taken and diluted and assayed using spectro-photometer (UV-1900, Shimadzu, Japan) at 289 nm which was the wavelength of maximum absorption. The content of the active ingredient was then determined using a reference Beer's plot prepared at 289 nm.

Dissolution profiles studies

Dissolution studies were then carried out using the dissolution apparatus with paddle, (U.S.P. model 2, Erweka, Germany), at 50 rpm with 0.1 N HCl as the dissolution medium. A 900 ml volume of the dissolution medium was measured into the dissolution apparatus maintained at 37.0±1.0°C. The machine was allowed to equilibrate for 30 min after which one tablet was introduced and allowed to sink to the bottom of the flask before the process was started. A 5 ml volume of the dissolution medium was withdrawn after every 5 min and replaced with 5 ml of fresh 0.1 N HCl for a period of 60 min. The samples withdrawn were then determined spectrophotometrically using ultra violet spectrophotometer model (UV-1900, Shimadzu, Japan).

Evaluation of the tablets for hypoglycemic properties and effect on some biochemical parameters

Male wistar rats were rendered diabetic by single intra-peritoneal injection of freshly prepared alloxan monohydrate solution (150 mg/kg body weight) and allowed free access to water and food for one week. On the 8th day the blood glucose levels of the animals were checked and animals with blood glucose levels greater than 180 mg/dl were selected for the study. Each batch of the prepared tablets was administered per oral to a group of five animals separately at the dose of 100 mg/kg per day for 21 days with glibenclamide and distilled water as positive and negative controls respectively. The blood glucose levels were measured at predetermined time for each group including the control groups for 21 days. Measurement of blood glucose level was carried out with Accu- check® advantage glucometer (Roche Diagnostics, Mannheim Germany) with readability of 10.8-599.4 mg/dl (0.6-33.3 mmol/l).

At the end of the 21 days, blood was collected from each animal from retro bulbar plexus through the median canthus of the eye. Blood of each animal was used to determine blood parameters such as aspartate aminotransferase (ASP), alanine amino-transferase (ALT), alkaline phosphatase (ALP), total cholesterol, and total triglyceride using commercial kits from Randox Laboratories U.K. The activities of catalase (CAT) enzyme, reduced glutathione (GSH) and the concentration of malondialdehyde (MDA) were also determined.

Statistical analysis

IBM Statistical Package for Social Sciences (SPSS) software version 21 was used to analyze data collected. One way analysis of variance (ANOVA) was used to determine statistical significance among groups. Mean values were separated using Duncan multiple comparison test, and values were considered significant if the p value was less than 0.05.

RESULTS AND DISCUSSION

Micromeritic properties of granules

The micromeritic properties of the batches of the granules were shown in Table 2. The values of the Carr's compressibility index and Hausners quotients (HQ) show that they have good flow and packing properties even though the angles of repose did not show correlation with other indices of flow. There was general increase from bulk to tapped density as a result of densification of the granules brought about by tapping. The bulk densities and the tapped densities ranged from 0.40 to 0.62 g/ml. The Hausner quotient ranged from 1.02 to 1.36 while the Carr's compressibility index ranged from 2.04 to 26.67%. HQ values ≤ 1.25 represent good flowing granules. The values of HQ for batches D2 and A3 slightly fell outside 1.25 as shown in Table 2. These correlated with their CI values of 20.83 and 26.67%, respectively. All the granule batches exhibited good flow properties which ranged from 11.04 to 22.47 g/s. The flow rate and the angle of repose did not follow any particular trend and did not correlate with HQ and CI values. Overall, all the granule batches are expected to flow uniformly from tablet press hopper to the die cavity producing tablets of uniform weight and content of active ingredients.

The particle size distributions of the granules are shown in Figure 1. The batches show particle size distribution of 65 -75% by weight lying between 425 – 840 µm. Particle size distribution is evaluated at developmental stages to establish appropriate particle size for drug product quality control. Powders are known to be compressible from 250 to 1000 µm (Liebermann et al., 1989). The particle size distribution of drug-excipients blend affect factors such as flowability, blend uniformity, and compatibility which has influence on the safety, efficacy and quality of the product (Snorek et al., 2007).The physicochemical properties of tablets such as thickness, appearance, friability, weight, disintegration time, dissolution time and potency were found to be affected by particle size distribution (Amin et al., 2014). Also, Echie and Kudehinbu (2009) observed that increase in granules size for example produces increase in the packing fraction of the granules. This implies that there will be a higher degree of consolidation of the particles due to greater tendencies of larger particles deform and fragment easily resulting in larger number of bonding units. Food and Drug Administration (FDA) recommended particle size of granules as a means of demonstrating equivalence between batches (FDA, 1994).

Table 2. Micromeritic properties of the herbal formulations.

Batch	Bulk density (g/ml)	Tapped density (g/ml)	Haus.Quot (HQ).	Carr's index (%) (CI)	Angle of repose (°)	True density (g/ml)	Flow rate (g/s)
A_1	0.56 ± 0.009	0.59 ± 0.011	1.05	5.08	40.08 ±1.62	1.029	19.12 ± 0.84
B_1	0.40 ± 0.005	0.48 ± 0.009	1.20	16.67	57.24 ± 3.57	1.080	11.04 ± 0.96
C_1	0.42 ± 0.005	0.51 ± 0.009	1.21	17.64	42.38 ± 1.35	1.067	16.01 ± 0.82
D_1	0.50 ± 0.005	0.55 ± 0.011	1.10	9.09	39.25 ± 2.06	1.093	22.47 ± 0.41
A_2	0.52 ± 0.005	0.60 ± 0.011	1.15	13.33	41.83 ± 1.20	1.005	18.31 ± 0.76
B_2	0.45 ± 0.008	0.51 ± 0.008	1.13	11.76	45.72 ± 1.07	1.168	12.56 ± 0.64
C_2	0.49 ± 0.007	0.54 ± 0.11	1.10	9.25	39.54 ± 1.48	1.067	17.97 ± 0.57
D_2	0.57 ± 0.005	0.72 ± 0.011	1.26	20.83	41.83 ± 1.59	1.054	20.20 ± 0.94
A_3	0.44 ± 0.007	0.60 ± 0.012	1.36	26.67	41.82 ± 1.07	1.003	21.10 ± 1.05
B_3	0.43 ± 0.007	0.47 ± 0.008	1.07	6.38	39.87 ± 1.25	1.108	15.83 ± 0.64
C_3	0.48 ± 0.012	0.49 ± 0.013	1.02	2.04	39.76 ± 2.64	1.041	21.70 ± 0.84
D_3	0.62 ± 0.011	0.69 ± 0.017	1.11	10.14	43.44 ± 0.52	1.029	19.11 ± 0.77
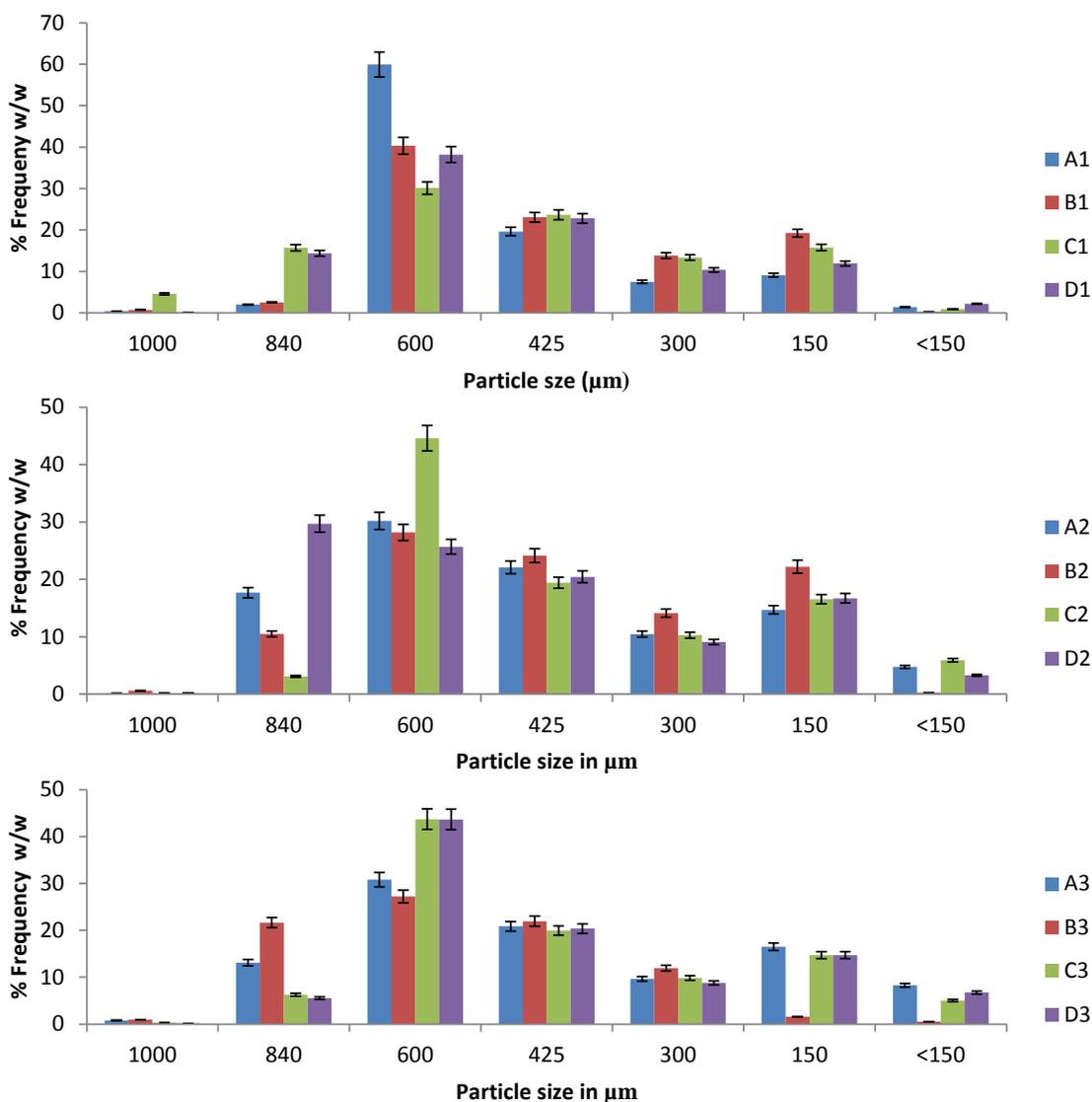
Figure 1. Particle size distribution of the different batches of *A. cepa* tablets.

FTIR spectrum of *A. Cepa* aqueous extract

FTIR spectrum of batch A1

FTIR spectrum of batch B2 granules

FTIR spectra of batch C3 granules
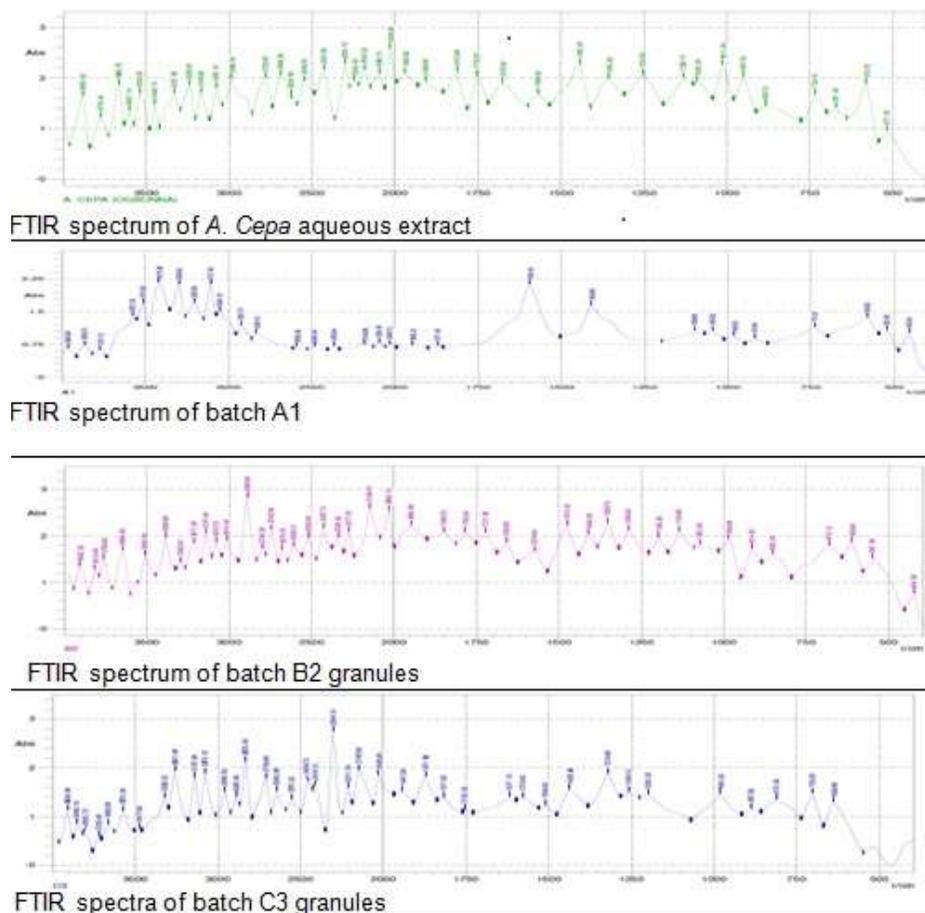
Figure 2. FTIR spectrum of the A. cepa extract, batches A1, B2 and C3.

Drug/excipients compatibility

FTIR

The FTIR spectra of the extract, excipients used and the formulations suggest that the extract was compatible with the excipients used. The extract was found to be compatible with the excipients used to formulate the tablets as there were no additional peaks or new functional groups in the different batches of the formulations as shown in Figure 2. Functional groups found in the extract include 1675.00 cm$^{-1} \rightarrow$ C=C (aromatic), 1752.20 cm$^{-1} \rightarrow$ C=O and 1813.96 cm$^{-1} \rightarrow$ C=O (esters). These were found compatible with formulation batches B_2 (1659.56, 1721.32, 1848.70 cm^{-1}), A_1 (1408.66, 1593.94 cm^{-1}) and C_3 (1617.30, 1752.20, 1817.82 and 1871.86cm^{-1}) which were selected as representatives of all the batches. These indicate absence of interaction between the extract and the excipients.

Tablet properties

The herbal tablets were of good physical properties,

compact and hard as shown by the values of different tablets properties shown in Table 3. The hardness and friability values were within the limits that ensures adequate strength for the tablets. These are indicated by low friability (0.033 - 0.36%), hardness within the range of 4.73±0.63 - 6.68±0.51 mg and low variation in weight (293.15±7.21 - 308.20±5.66 mg). The batches complied with the United States Pharmacopoeia (USP) (2007) official requirements for uniformity of weight and drug content. Drug content was within 95.56±1.08 – 101.43±1.33% in all the batches as shown in Table 3.

The batches containing gelatin and PVP as binders had lower disintegration time. Most of the batches disintegrated within 30 min. Those with maize starch and CMC as binder respectively had higher disintegration time even though they are within the limit allowed for herbal tablets in Pharmacopoeia Committee of Peoples Republic of China (1995). Disintegration time test is a relative measure of tablet hardness which is dependent on many formulation factors such as binder concentration and type, type and concentration of disintegrants, compression pressure, lubricant type and concentration, particle size of the granules (Banker and Rhodes, 1979). Wet granulation method of tablet preparation has been

Table 3. Tablet properties of *A. cepa* herbal tablets.

Batch	Hardness (KGF)	Friability (%)	Weight variation (mg)	Disintegration time (min.)	Content of API (%)
A_1	6.37 ± 0.75	0.25	297.15 ± 7.96	15.07 ± 1.34	96.53 ± 0.05
B_1	5.05 ± 0.91	0.033	296.97 ± 9.00	11.7 ± 3.37	96.33 ± 1.02
C_1	5.58 ± 0.79	0.15	296 ± 9.10	39.00 ± 4.05	95.84 ± 0.58
D_1	5.71 ± 0.58	0.42	300.75 ± 5.63	23.87 ± 2.98	98.65 ± 0.48
A_2	5.91 ± 0.69	0.12	301.85 ± 6.92	14.34 ± 2.27	100.21 ± 0.96
B_2	4.73 ± 0.63	0.36	300.05 ± 8.54	14.95 ± 2.04	99.04 ± 0.028
C_2	5.59 ± 0.56	0.28	293.15 ± 7.21	21.5 ± 4.51	95.56 ± 1.08
D_2	6.68 ± 0.51	0.16	295 ± 5.63	18.80 ± 8.15	95.12 ± 0.35
A_3	6.07 ± 0.57	0.31	300.90 ± 6.97	13.16 ± 6.38	98.60 ± 0.45
B_3	4.95 ± 0.67	0.28	296.9 ± 8.69	14.36 ±2.24	96.05 ± 0.77
C_3	5.16 ± 0.54	0.18	298.55 ± 8.10	30.83 ±5.49	97.23 ± 0.42
D_3	6.51 ± 0.58	0.22	308.20 ± 5.66	19.03 ±1.30	101.43 ± 1.33
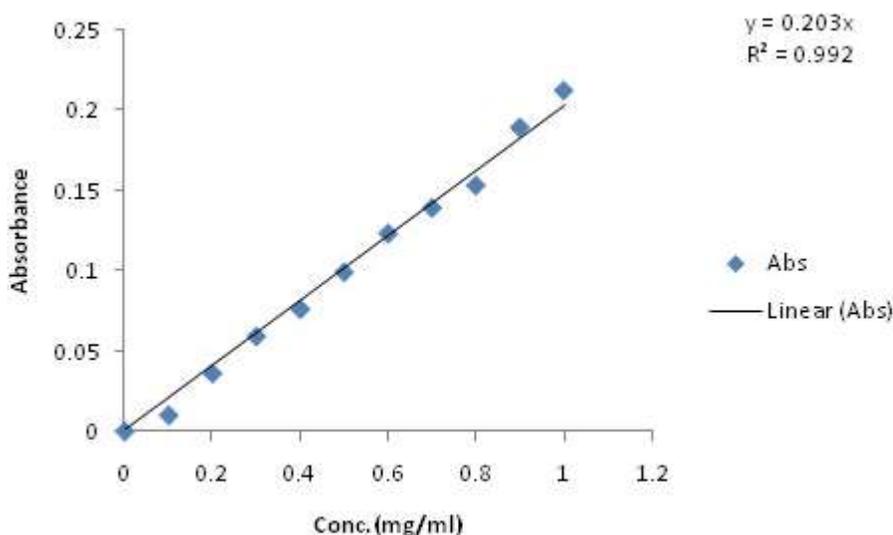
Figure 3. Beer's calibration curve of *A. cepa* L. aqueous extract in 0.1N HCl.

shown to produce strong tablet without significant effect on drug release (Odeku and Fell, 2006; Kondetti et al., 2014).

In vitro dissolution studies showed good drug release properties as cumulative drug release were between 75.37 and 115.27% for all the batches as shown in Figure 4. Highest drug release was seen in batch A_1 with drug release of 115.27%, while batch D_2 had the least drug release of 75.37% even though other batches D_1 and D_3 also prepared with maize starch as the binder had better drug release than those prepared with CMC as the binder (C_1- C_3). The absorption spectrum for *A. cepa* showed a maximum at 289 nm and was found to obey Beer's law at a concentration range of 0.1 - 0.9 mg/ml as shown in Figure 3. Batches B_1, B_2, B_3 and D_1 released up to 90% of their drug within 30 min, while batches A_1, A_2, and D_3 released up to 90% of their drug in 45 min. Batches C_1, C_2, C_3 and D_2 could not release up to 90% within 60 min. The results of the release studies show that with respect to binders the order of drug release was PVP > gelatin > maize starch > CMC. Tablets containing soluble binder such as PVP and gelatin have been shown to possess high dissolution rate, whereas, slow and incomplete dissolution is associated with starch paste as binder. Polymers such as CMC increases disintegration time and slow down dissolution (Odeniyi et al., 2006).

Anti-hyperglycemic effect of the formulations

The *in vivo* anti-hyperglycemic effects were higher in batches A_1, A_2, A_3, B_3, C_1, and D_3. Maximum blood glucose reduction of 12.33 to 70.96% was attained by the batches of tablets administered as shown in Figure 5.
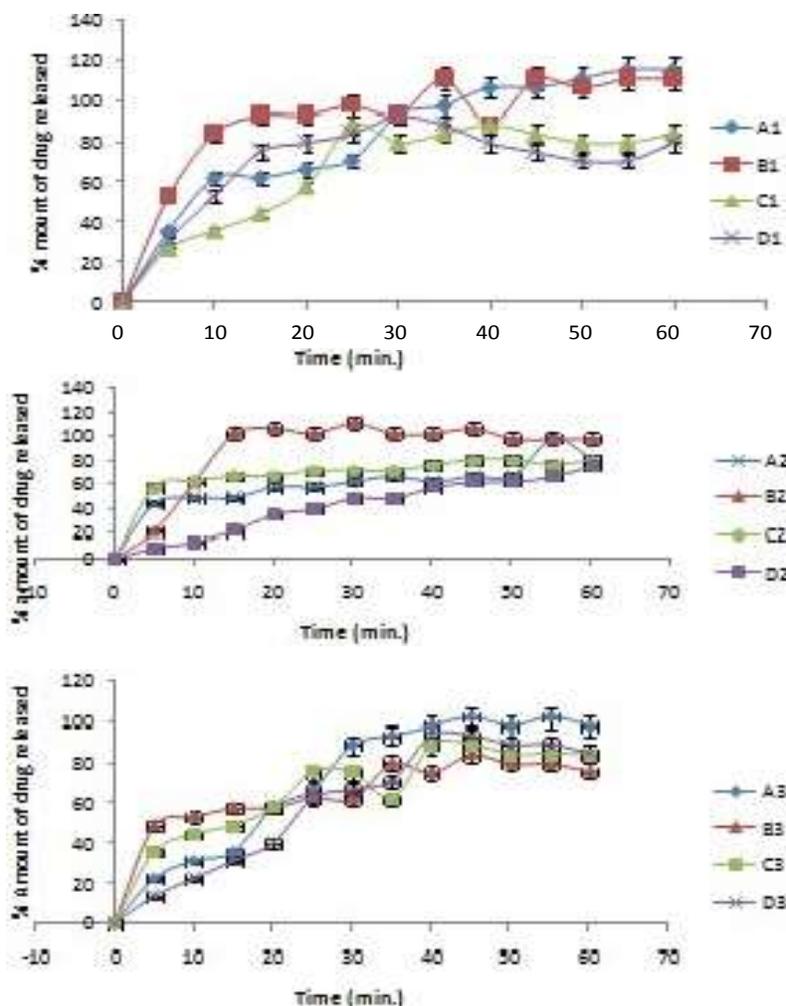
Figure 4. Dissolution profiles of the different batches of the *A. cepa* tablet.

Batch A_1 achieved the highest blood glucose reduction of 70.96% while batch D_1 had the lowest reduction of 12.33%. The difference in the hypoglycemic effect was a combination of excipients used and the release profiles of the tablets. Various formulation factors such as type and concentration of disintegrants, binders, lubricants and hardness or compression force do affect drug release from tablets. A complex relationship exists between *in vitro* dissolution or performance of a drug and *in vivo* performance, and this depends on the characteristics of the specific drug molecules, product design and *in vitro* test conditions (Lin et al., 2016).

Effect on some biochemical parameters

The effects of oral administration of the tablets on the plasma liver enzymes activities, plasma anti-oxidant biomarker activities and on plasma lipid profile indicate significant activities ($p < 0.05$) of the herbal tablets in some batches compared to the negative control as shown in Table 4. Batches A_1, A_2, A_3, B_3, C_1 and D_3 were more effective in reducing the activity of ALP than other batches. This could be due to the different excipients used in preparing the batches which affected drug release and absorption differently. The same trend was observed in AST and other biochemical parameters. In batches B_1, B_2, B_3 and D_1, D_2, D_3 prepared with the binder polyvinyl pyrrollidone and maize starch respectively, those containing Ac-Di- Sol® as the disintegrants were more effective than those prepare with Primogel® and maize starch as disintegrants. However, batch B_1 was found not be significantly different from the negative control in all the parameter tested for unknown reason. The batches prepared with the binder sodium carboxylmethyl cellulose (C_1, C_2, and C3) generally were less effective than the other batches. Unlike the other batches, the batch with Ac-Di- Sol® as the disintegrant was found to be less effective than those with Primogel® or maize starch as disintegrant. It is possible that the disintegrant action of Ac-Di- Sol® was impaired in the presence of CMC.
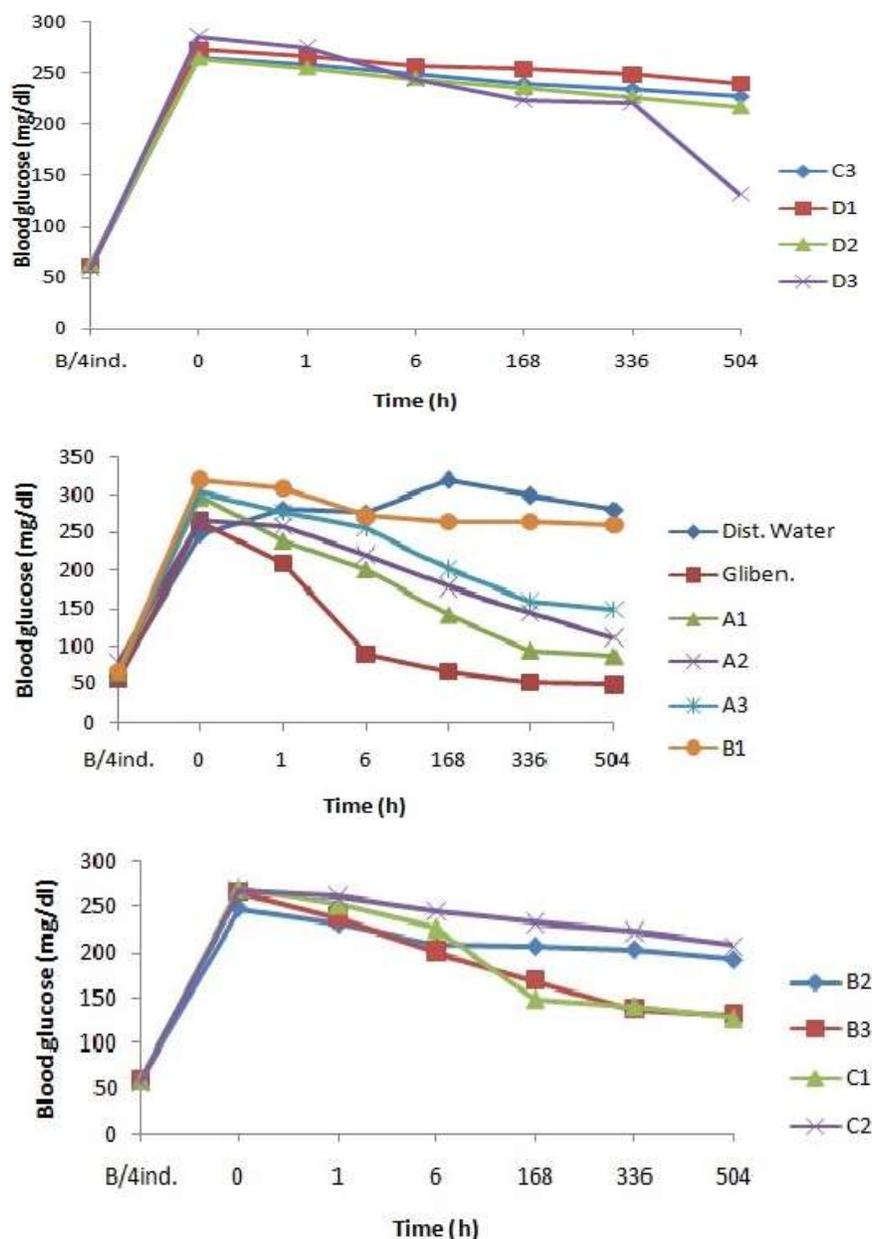
Figure 5. Effect of the tablets on blood glucose levels of the diabetic rat.

In batches A_1, A_2 and A_3, which were prepared with gelatin as binder, the batch containing maize starch as the disintegrant was found to be more effective than those with either Primogel® or Ac-Di- Sol® ($A_1>A_2>A_3$ and $C_1>C_2>C_3$. In all the batches Ac-Di- Sol® containing tablets were found to be more effective when the binder used was PVP or maize starch. Conversely maize starch was a better disintegrant when gelatin or CMC was used as the binder.

Conclusion

Evaluation of the formulations for antidiabetic properties

and effects on some biochemical parameters show that the formulations did not produce equal effects on the blood glucose and other blood biochemical parameters. This could be due to difference in drug release and absorption following oral administration of the tablets. However, batches A_1, A_2, A_3, B_3, C_1 and D_3 were found to be more effective *in vivo* than the other batches.

Aqueous extract of *A. cepa* can therefore be formulated into tablet dosage form using wet granulation. However, with the wet granulation method, the use of sodium carboxy methyl cellulose as a binder at 5% (w/w) gave tablets with relatively poor release and disintegration properties. It was found that some disintegrants are less

Table 4. Effect of *A. cepa* tablets on some blood biochemical parameters.

Batch	ALT (I.U./l)	AST (I.U./l)	ALP (I.U./l)	Tot. Chol. (mg/dl)	Tot. Trig. (mg/dl)	CAT.(U/ml)	GSH (U/ml)	MDA (nmol/ml)
A_1	86.87±1.46	64.25 ±1.29	69.73±4.05	81.57±1.85	87.30±0.70	0.783±0.025	12.33±0.25	0.333±0.023
A_2	93.13±1.99	66.43±1.73	68.57±3.20	83.10± 1.18	92.47±2.83	0.720±0.017	11.7± 0.40	0.327±0.015
A_3	95.83±0.45	73.68± 2.18	83.13±1.45	91.13 ±1.91	98.07±1.46	0.626±0.012	9.10±0.30	0.360 ± 0.02
*B_1	148.73±3.23	126.97±1.68	121.10±1.4	125.20±1.31	130.40±1.3	0.426±0.021	3.30±0.16	1.367±0.115
B_2	108.67±11.31	93.11±24.28	102.63±11.76	124.23±4.82	125.40±5.36	0.577±0.123	5.57±0.12	0.607±0.263
B_3	91.87±2.50	66.40±1.03	73.87±2.98	82.10±1.83	91.20±1.80	0.77±0.040	8.67±0.15	0.373±0.112
C_1	97.23±2.21	72.87±1.77	77.40± 5.47	88.60± 6.39	95.73±2.71	0.737±0.031	8.17±0.25	0.413±0.025
C_2	92.9±1.37	90.97±2.69	89.07±1.89	122.67±1.25	130.9±0.96	0.553±0.015	6.57± 0.35	0.720±0.017
C_3	96.33±0.40	95.58 ±1.52	80.47±2.26	124.83±0.25	133.77±0.40	0.59±0.020	5.47±0.35	0.760±0.017
D_1	101.5± 2.81	102.66±2.99	109.53±1.59	120.13±0.85	130.73±0.65	0.49±0.017	4.5± 0.17	0.917±0.025
D_2	97.87±179	92.18±5.40	86.87±1.72	120.57±0.55	128.93±1.35	0.493±0.030	4.67±0.12	0.857±0.057
D_3	94.73±3.16	68.54±2.80	77.93±0.96	96.97±1.17	103.23±1.34	0.743±0.031	7.4±0.3	0.557±0.050
*Neg	154.5± 4.15	99.41±7.56	125.63±1.24	127.76±1.21	130.67±0.77	0.457±0.031	4.04±0.06	1.094±0.072
Glib	84.21±1.23	128.5±2.01	61.5±3.78	81.80±2.30	86.6±0.62	0.757±0.032	12.37±0.15	0.366±0.004

effective when used with some binder in preparation of tablets.

Since herbal preparations play important roles in the management of diabetes mellitus because of their bioactive constituents, more research efforts are however needed to standardize herbal preparations on the basis of their active constituents, understand their mode of action, dosage and safety.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

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