

Evaluation of the Anti-Diabetic Potential of the Methanol Extracts of *Aloe camperi*, *Meriandra dianthera* and a Polyherb

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Abstract

The objective of the study was to evaluate the anti-diabetic activities of methanol extracts of *Aloe camperi* (AC), *Meriandra dianthera* (MD) and a polyherbal drug (PH) in diabetes induced Wistar albino rats. A single dose of alloxan monohydrate (150 mg/kg, i.p.) was used to induce diabetes mellitus (DM). Diabetes was confirmed by the elevated blood glucose levels determined after 72 h of induction. Animals with mean fasting blood glucose (FBG) level more than 200 mg/dl were recruited for the experiment. The herbal extracts at doses of 200 and 400 mg/kg and standard drug-metformin (5 mg/kg) were administered orally to the diabetic rats for 21 days and the FBG level was estimated on 0, 7, 14 and 21 days. The herbal extracts showed dose-dependent fall in FBG levels and the result exhibited very significant ($P < 0.001$) decreases in FBG level by the end of the experimental day as compared to the diabetic control. The highest antihyperglycemic effect was observed by MD extract at 400 mg/kg and was comparable to the standard drug. Oral glucose tolerance test (OGTT) was also conducted on normal rats and thus glucose at 2 g/kg per body weight was loaded via oral gavage to all groups 30 min after extract administration. All the groups showed significant increase ($P < 0.01$ or $P < 0.05$) in FBG level at 30 min following glucose loading. The hyperglycemia with glucose challenge was significantly brought down ($P < 0.001$) by all herbal extracts at 60 and 120 min relative to the negative control. Moreover, acute oral toxicity tests was conducted based on the protocols of OECD-425 and thus the LD₅₀ of the herbal extracts was estimated to be greater than 2000 mg/kg. Statistical analysis was performed using One-Way ANOVA followed by Dunnett's test for multiple comparisons, and values of $P < 0.05$ were considered as statistically significant.

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Keywords

Antihyperglycemia, Fasting Blood Glucose Level, *Aloe camperi*, *Meriandra dianthera*, Polyherb

1. Introduction

Diabetes is a complex and chronic illness characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The two common types of diabetes are known as type 1 diabetes (due to β -cell destruction, usually leading to absolute insulin deficiency) and type 2 diabetes (due to a progressive insulin secretory defect on the background of insulin resistance) [1]-[4].

The incidence of diabetes especially type 2 diabetes mellitus (T2DM) is rapidly growing in the world. Diabetes has become one of the major causes of premature illness and death in most countries, mainly through the increased risk of cardiovascular diseases [5] [6]. Diabetes is a leading cause of blindness, amputation and kidney failure. These complications account for much of the social and financial burden of diabetes. Worldwide, 3.2 million deaths are attributable to diabetes every year. The number of people with diabetes was 387 million for 2014 and would be about a double in the next 30 years, to reach a total of 592 million by 2035 [7]-[9]. Although diabetes is sometimes considered a condition of developed nations, the loss of life from premature death among persons with diabetes is greatest in developing countries [10] [11]. It was estimated that 19.8 million people had diabetes in Africa in 2013 and that would rise to 41.4 million by 2035 [12]. In Eritrea, the DM prevalence of adults was estimated to be 4.89% for 2014 [13].

There are many synthetic anti-diabetic agents currently available; however, these have a number of adverse effects on the body [14]. Currently, medicinal plants continue to play an important role in the management of DM, especially in developing countries; plant products have less toxicity and their use is cost-effective than conventional medicines. Frequently, however, it is mandatory to provide scientific proof in order to justify the use of a plant or its active components [15]-[20]. *In vivo* experimental models have been used to screen the anti-diabetic efficacy of different natural products that display several effects besides lowering blood glucose. In view of the lack of parallel studies of their toxicity, these experiments are considered a screening step in the search of drugs for the treatment of diabetes [21] [22].

The plants of interest for this study were selected based on the ethnobotanical survey and preliminary phytochemical screening conducted recently [23]. These plants include *Aloe camperi* (AC), *Meriandra dianthera* (MD) and a Polyherb (PH) prepared from the seeds of *Lepidium sativum* (LS), *Brassica nigra* (BN) and *Nigella sativa* (NS). *A. camperi* is a species of aloe native to Eritrea and Ethiopia; it is so far not known anywhere else. The specific epithet “*camperi*” was, according to Schweinfurth, given in honour of “an esteemed friend Manfredo Camperio, who did so much for the Italian Colony of Eritrea”. The species was described in 1894 based on the type material collected near Ghindae in Eritrea. *A. camperi* is distinguished from the related species of the clavate perianth which is 18 - 22 mm long and the small bracts $2 - 3(-5) \times 1-2$ mm. It grows abundantly on rocky slopes and sandy alluvial plains along the eastern escarpment between 550 and 2700 m. The main flowering period is from March to May [24] [25].

M. dianthera is a genus of plants in the Lamiaceae family; it is native to Eastern Africa, the Arabian Peninsula, and India. *M. dianthera* is a fragrant shrub 50 cm to 2 m with dense branches; found in open bush vegetation sometimes cultivated; 1800 - 3000 m. Most parts covered with short grey hairs give a white appearance. The leaves are very aromatic, long oval, 3.5 - 7.0 cm, the blade narrows at the base into winged stalk less than 2 cm, midrib clear below, leaf edge finely round-tooth, the tip more or less pointed; both surfaces hairy, more dense below [26] [27].

As shown in **Figure 1**, both *A. camperi* and *M. dianthera* are coniferous species and widely distributed in Central and Southern Zones of Eritrea [28]. These plants have wider applications in the treatment of diabetes and other ailments in the traditional medical practices of the communities of these Zones. From the previous phytochemical analysis, the methanol extracts of the plants contain alkaloids, phenols, saponins, flavonoids and other secondary metabolites were confirmed that [23]. Recently, it was reported that the leaves of *M. dianthera* were used for hypertension and diarrhoea in Southern Tigray, Ethiopia [29].

The current study involves *in vivo* anti-diabetic screening of the methanol extracts of *A. camperi*, *M. dianthera*

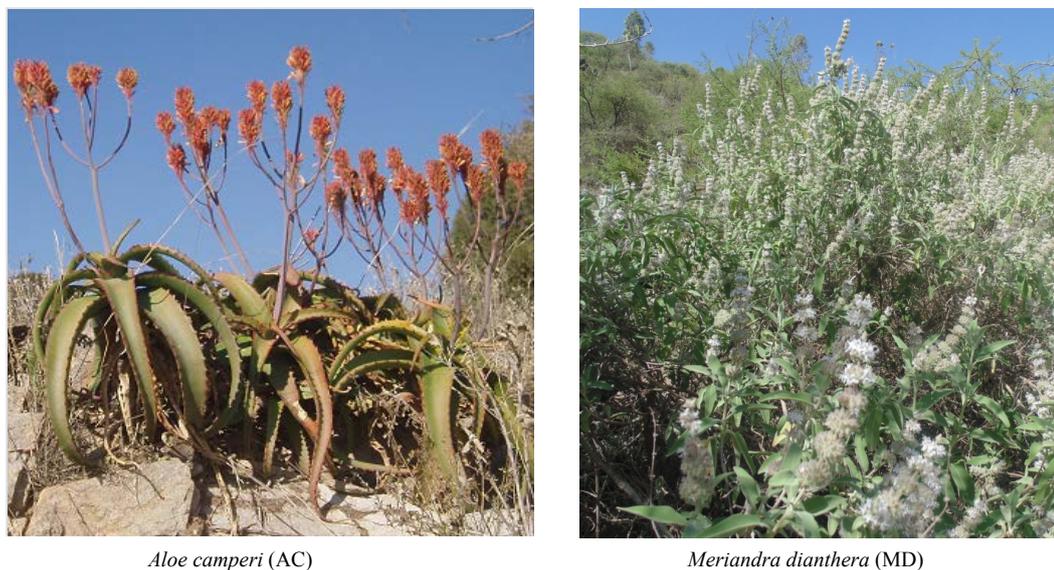


Figure 1. Photos of *A. camperi* and *M. dianthera* taken in the vicinity of Adi-hawisha, Maekel Zone, Eritrea.

and a Polyherb. Based on literature survey, the anti-diabetic efficacy of these herbal extracts has not yet been reported.

2. Materials and Methods

2.1. Drugs and Reagents

Alloxan monohydrate was purchased from Sigma (St. Louis, MO, USA; stored at 4°C) and Metformin was obtained from Cipla Pharmaceuticals, Kenya. Glucometer (GOD/POD) diagnostic Kit and the glucometer strips were purchased from Doxpan Venture, Nairobi, Kenya. Other chemicals used were of analytical grade.

2.2. Plant Materials and Extraction

The fresh leaves of *A. camperi* and *M. dianthera* were collected from Adi-hawisha between the months of November 2014 and December 2014. The components of the Polyherbal extract (dry seeds of LS, BN and NS) were bought from herbal shop in Asmara, Eritrea. Taxonomic identification was done and voucher specimens were deposited at the Herbarium of the Eritrea Institute of Technology, Eritrea.

The leaves of *A. camperi* and *M. dianthera* were washed with distilled water and dried under a shade at room temperature. The dried leaves and seeds of the plants were powdered using an electric blender and filtered using 35 mesh size (200 μ m) sieve. Each powdered sample (100 g) of *A. camperi*, *M. dianthera* and the Polyherb were defatted with light petroleum (60°C - 80°C) and then macerated in 1000 ml of methanol for five days with occasional stirring. Similarly, the PH extract was prepared by mixing equal proportion of LS, BN and NS. The methanol extracts were evaporated *in vacuo* at 50°C and the concentrated extracts were stored in air tight containers at 4°C until further use.

2.3. Experimental Animals

Wistar albino rats (150 - 170) of 8 - 10 weeks of either sex were obtained from Kabete Veterinary Farm, Nairobi, Kenya. All the experiments were conducted in the Animal House of Jomo Kenyatta University of Agriculture and Technology (JKUAT). After randomized grouping and before initiation of the experiment, animals were housed, for two weeks, in ventilated polypropylene cages under standard laboratory conditions of temperature and humidity to acclimatize. During this period, the rats were given standard laboratory diet, in the form of rat pellets from *Unga Feeds Limited*, Nairobi, Kenya, and water was allowed *ad libitum*. Twelve hour light-dark cycle was also allowed in the entire period of the experiment [30] [31]. The experimental protocols were approved by Institutional Animal Ethics Committee of JKUAT.

2.4. Acute Oral Toxicity Test (AOTT)

Acute oral toxicity study was performed as per the protocols of Organization for Economic Cooperation and Development (OECD) guidelines 425 [32]. Nulliparous female animals were fasted overnight prior to dosing. The fasting body weight of each animal was determined and the dose was calculated accordingly. The crude methanol extracts (2000 mg/Kg) of *A. camperi*, *M. dianthera* and the Polyherb were administered in a single dose by gavage. The rats were then kept under strict observation for physical and behavioural changes for 24 h, with special attention during the first 4 h. Following the results from the first rat, other four rats were recruited and fasted overnight and administered a single dose of 2000 mg/kg and were observed in the same manner. These observations continued for further 14 days for any signs of overt toxicity. Hence, 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of this dose were adopted for further antihyperglycemic studies.

2.5. Oral Glucose Tolerance Test (OGTT)

OGTT was assessed, according to standard methods, to the overnight fasted rats [33] [34]. The Wistar albino rats were divided into 8 groups of 6 rats each; the aim was to study the effect of plant extracts on changes in blood glucose level in normal rats. The three plant extracts and standard drug (metformin) were administered as shown in **Table 1**. Thereafter, following 30 min post extracts and standard drug administration, glucose solution (40% aqueous solution) at 2 g/kg per body weight was loaded via oral gavage to all the groups. Blood was obtained from tail vein using sterile needle and the changes in blood glucose levels were measured, using a glucometer (On Call Plus, ACON Laboratories, Inc., USA) and recorded after 30 min of treatment (considered as 0 min) and after 30, 60, and 120 min of glucose loading.

2.6. Antihyperglycemic Activity

A single dose of Alloxan at 150 mg/kg (as a 5% solution) was prepared using normal saline (0.9% NaCl) at room temperature and immediately administered intraperitoneally to the rats. Animals were then kept for the next 24 hours on 10% glucose to prevent hypoglycaemia. Diabetes was confirmed after 72 h of Alloxan injection, the blood samples were taken from tail vein and plasma glucose levels estimated with the help of a Glucometer using strip method. The rats with fasting blood glucose level higher than 200 mg/dl were included in the study [35] [36].

The plant extracts were administered orally for 3 weeks using gavage. Starting from the 1st day (3rd day of Alloxan injection) of extract administration to diabetic rats, FBG level was measured in the 4th, 7th, 14th and 21st days. As indicated in **Table 1**, the experiment was carried on 9 groups of each six rats; the animals were grouped in a similar way as OGTT experiment except that there was a diabetic control group. The body weights of all the rats were measured pre-treatment (*i.e.* after 3 days of diabetes induction) and end of the treatment (*i.e.* after 21 days of treatment) by using digital weighing balance. The relationship in body weight of each group versus the activity of the extracts was thus investigated.

Table 1. Grouping of animals for OGTT and antihyperglycemic experiments.

Group label	Description
NC	Normal control, received normal saline (0.9% NaCl in distilled water)
PC	Administered 5 mg/kg body of metformin
DC*	Diabetic control, received 150 mg/kg Alloxan
AC200	Administered 200 mg/kg body weight of AC
AC400	Administered 400 mg/kg body weight of AC
MD200	Administered 200 mg/kg body weight of MD
MD400	Administered 400 mg/kg body weight of MD
PH200	Administered 200 mg/kg body weight of PH
PH400	Administered 400 mg/kg body weight of PH

Key: NC: normal control (saline); PC: positive control (metformin); DC*: diabetic control (Alloxan) used for the antihyperglycemic studies only.

2.7. Statistical Analysis

All data collected was first stored using Microsoft Excel and expressed as mean \pm SEM. A two tailed paired t-test was used first to determine any significant difference between baseline and intervention. Further statistical analysis was then performed using One-Way ANOVA in SPSS software for Windows version 21.0, followed by Dunnett's test for multiple comparisons, and values of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Acute Oral Toxicity Test

The animals were safe up to a maximum dose of 2000 mg/kg per body weight for all the three extracts. There were only temporary changes in the normal behavioural pattern of the rats administered with *A. camperi* and *M. dianthera*; however there were no signs and symptoms of acute toxicity and mortality when fed with all the extracts. The results confirm that the three plant extracts have LD₅₀ value greater than the test dose (2000 mg/kg).

3.2. Oral Glucose Tolerance Test

The OGTT effects of the extracts of *A. camperi*, *M. dianthera* and the Polyherb are summarized in **Table 2**. Administration of *A. camperi*, *M. dianthera* and a Polyherb at doses of 200 and 400 mg/kg body weight to the glucose loaded rats showed significant reduction in blood glucose levels. Post to the extracts' and standard drug administration (0 min), there were no significant difference in BGL among all the groups ($P > 0.05$). All groups, however, showed significant increase ($P < 0.01$ or $P < 0.05$) in BGL 30 min following glucose loading, confirming the induction of hyperglycemia. Compared to the normal control all groups, except PH200, showed significant difference (PC and MD400 with $P < 0.001$, AC400 and MD200 with $P < 0.01$, AC200 and PH400 with $P < 0.05$) in BGL 30 min following glucose loading.

On the other hand, in the inter-group analysis, hyperglycemia with glucose challenge was significantly ($P < 0.001$) brought down with PC, AC400, MD200, MD400, and PH400 at 60 min relative to the negative control. AC200 and PH200 also showed significant ($P < 0.01$) decrease in BGL at 60 min. At 120 min, a significant difference (mostly $P < 0.001$) was achieved from the groups compared to the negative control. Moreover, at 120 min, a significant difference (PC, MD200, MD400 with $P < 0.001$, AC200, AC400 with $P < 0.01$ and PH200, PH400 with $P < 0.05$) was observed compared to the peak hyperglycemia (BGL level at 30 min).

3.3. Antihyperglycemic Effect

The decline in blood glucose level is generally used as the main directory for antihyperglycemic effect of drugs. Thus, treatment with methanol extract of the plants has proved highly effective in causing significant antihyperglycemic response in the experimentally diabetic rats. As shown in **Table 3** and **Figure 2** and **Figure 3**, a very

Table 2. Effect of the methanol extracts of *A. camperi*, *M. dianthera* and the Polyherb on oral glucose tolerance test conducted on normoglycaemic rats.

Group	Blood glucose level (in mg/dl)			
	0 min	30 min	60 min	120 min
NC	98.28 \pm 2.70	117.77 \pm 1.71 ^{a2}	114.52 \pm 1.49	112.21 \pm 1.70
PC	97.36 \pm 1.51	105.49 \pm 1.48 ^{a1}	99.04 \pm 1.32 ^{c3}	85.67 \pm 1.20 ^{b3,c3}
AC200	98.54 \pm 1.68	110.40 \pm 1.73 ^{a2}	105.10 \pm 1.25 ^{c2}	98.52 \pm 1.56 ^{b2,c3}
AC400	97.32 \pm 1.43	107.57 \pm 1.75 ^{a1}	103.24 \pm 1.62 ^{c3}	93.68 \pm 1.33 ^{b2,c3}
MD200	97.04 \pm 1.22	108.07 \pm 1.73 ^{a2}	102.64 \pm 1.84 ^{c3}	92.70 \pm 1.90 ^{b3,c3}
MD400	97.92 \pm 1.67	105.65 \pm 1.97 ^{a1}	98.55 \pm 1.15 ^{c3}	86.21 \pm 1.96 ^{b3,c3}
PH200	98.28 \pm 2.32	111.25 \pm 1.52 ^{a2}	106.48 \pm 1.44 ^{c2}	102.29 \pm 1.79 ^{b1,c2}
PH400	98.64 \pm 2.57	109.71 \pm 1.96 ^{a1}	103.64 \pm 2.20 ^{c3}	99.56 \pm 1.71 ^{b1,c3}

Key: All data were expressed as mean \pm SEM; n = 6 in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. NC: normal control; PC-positive control; ¹ $P < 0.05$; ² $P < 0.01$; ³ $P < 0.001$; a: compared to 0 min; b: compared to 30 min; c: compared to NC.

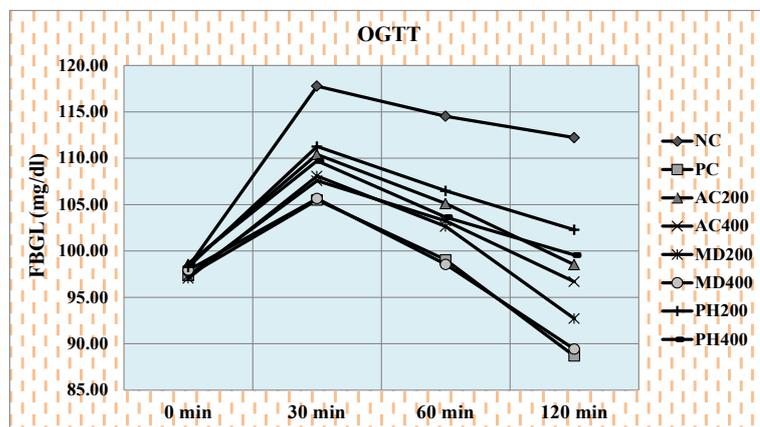


Figure 2. Effect of the methanol extracts of *A. camperi*, *M. dianthera* and the Polyherb on oral glucose tolerance test conducted on normoglycemic rats.

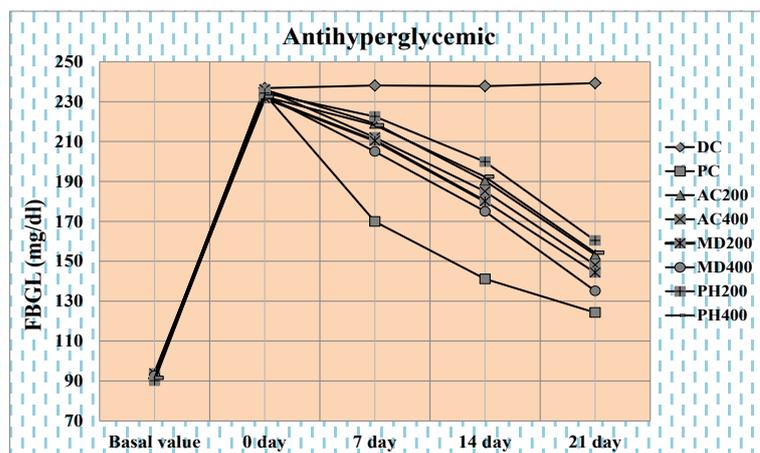


Figure 3. Antihyperglycemic Effect of the methanol extracts of *A. camperi*, *M. dianthera* and the Polyherb.

Table 3. Antihyperglycemic effect of methanol extracts of *A. camperi*, *M. dianthera* and the Polyherb.

Group	Blood glucose level (in mg/dl)			
	0 day	7 day	14 day	21 day
NC	88.2 ± 3.55	91.08 ± 3.40	90.72 ± 3.25	92.52 ± 4.21
DC	236.76 ± 3.84	238.08 ± 2.72	237.80 ± 2.54	239.28 ± 2.84
PC	233.80 ± 2.61	169.92 ± 4.78 ^{c3,a3}	141.12 ± 4.05 ^{c3}	124.28 ± 3.61 ^{c3,b3}
AC200	231.80 ± 2.93	210.44 ± 3.98 ^{c2,a1}	180.10 ± 3.28 ^{c3}	144.32 ± 3.58 ^{c3,b3}
AC400	233.16 ± 2.36	205.16 ± 3.52 ^{c3,a2}	175.19 ± 2.56 ^{c3}	135.12 ± 2.91 ^{c3,b3}
MD200	230.00 ± 3.37	210.44 ± 3.98 ^{c3,a2}	180.10 ± 3.28 ^{c3}	144.32 ± 3.58 ^{c3,b3}
MD400	233.16 ± 2.36	205.16 ± 3.52 ^{c3,a2}	175.19 ± 2.56 ^{c3}	135.12 ± 2.91 ^{c3,b3}
PH200	234.28 ± 2.60	222.56 ± 2.96 ^{c1,a1}	199.88 ± 3.74 ^{c3}	160.40 ± 4.03 ^{c3,b3}
PH400	232.44 ± 2.89	218.20 ± 1.82 ^{c2,a2}	192.33 ± 2.75 ^{c3}	154.28 ± 3.53 ^{c3,b3}

Key: All data were expressed as mean ± SEM; n = 6 in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. NC: normal control; DC: diabetic control; PC: positive control; ¹P < 0.05; ²P < 0.01; ³P < 0.001; a: compared to 0 day; b: compared to 7 day; c: compared to DC.

significant ($P < 0.001$) reduction in the blood glucose level was observed at the 7th day of the extracts administration of AC400, MD200, MD400 and metformin. Similarly, significant reduction in BGL was observed with AC200 ($P < 0.01$), PH400 ($P < 0.01$) and PH200 ($P < 0.05$) relative to the diabetic control. A very significant ($P < 0.001$) reduction in the blood glucose level was observed, by all of the crude extracts, at the end of 2nd week of treatment in the diabetic rats, which remained persistent up to the 3rd week of treatment.

Comparatively, each group showed significant reduction (AC200 and PH200 with $P < 0.05$; AC400, MD200, MD400 and PH400) in blood glucose level as compared to the first day of extract administration (0 day). Moreover, comparing the 21st day to 7th day, all the extracts showed a very significant ($P < 0.01$) reduction in BGL. By the end of the 21st day, the reduction of BGL of AC200, AC400, MD200, MD400, PH200, and PH400 was 35.05, 37.29, 37.74, 42.10, 31.53, and 33.63% respectively. Comparing the reduction brought about by the plant extracts to metformin (46.84%), it is evident that the reduction in BGL is quite comparable. After 21 days of treatment, the maximum reduction in BGL was observed in the rats treated with MD400 and minimum reduction in PH100 as compared to the other treatment groups.

Table 4 represents the effects of methanol extracts of *A. camperi*, *M. dianthera* and the Polyherb on the changes in body weight of normal control and treated diabetic rats.

Statistical analysis by One-Way ANOVA revealed that there was no significant difference among the groups during initial body weight estimation ($P > 0.05$). As shown in **Table 4**, a steady decrease in the body weight was observed in the diabetic control rats which was very significant ($P < 0.001$) by the end of the 3rd week of Alloxan treatment.

Administration of metformin to diabetic rats resulted in increase in the body weight compared to diabetic control rats; this suggests that metformin treatment has positive effect on maintaining body weight. However, diabetic rats treated with plant extracts at doses of 200 and 400 mg/kg per body weight showed decrease in body weight but it was significant improvement as compared to the body weight of the diabetic control group. Moreover, the methanol extracts of *A. camperi*, *M. dianthera* and the Polyherb showed significant change ($P < 0.05$) in body weight compared to the normal control group.

4. Discussion

It is believed that herbal products are safe and have been widely used worldwide for the treatment of diabetes. Therefore, extensive studies on the efficacy and safety of these herbs as alternative medicine in mandatory. This paper reports the evaluation of anti-diabetic activity of *A. camperi*, *M. dianthera* and a polyherb in experimental animal models after induction of diabetes by Alloxan monohydrate. Initially acute oral toxicity was evaluated and according to the OECD 425 protocol; if mortality is observed in one animal, then the same dose is repeated again to confirm the toxic dose. If mortality is observed in 3 animals out of five, then the dose administered is assigned as toxic dose. The herbal extracts proved to be safe with the limit dose; 2000 mg/kg per body weight [32].

Table 4. Body weight comparison of normal and treated diabetic rats.

Treatment group	Initial body wt (in grams)	Final body wt (in grams)	Change in body wt (%)
NC	163.35 ± 3.13	169.6 ± 2.97	6.25
DC	160.18 ± 3.27	130.05 ± 3.21 ^{***}	30.13
PC	161.06 ± 2.41	163.90 ± 2.49 ^{NS}	2.84
AC200	160.73 ± 3.39	148.01 ± 2.78 ^{**}	12.72
AC400	161.56 ± 3.07	150.23 ± 3.14 ^{**}	11.32
MD200	162.43 ± 2.85	152.06 ± 2.99 [*]	10.38
MD400	161.57 ± 3.10	155.54 ± 3.14 [*]	6.04
PH200	161.89 ± 2.75	136.86 ± 3.12 ^{**}	25.03
PH400	162.70 ± 3.48	141.70 ± 3.21 ^{**}	21.00

Key: All data were expressed as mean ± SEM; n = 6 in each group. Statistical significant test for comparison was done by two tailed paired t-test. NC: normal control; DC: diabetic control; PC: positive control; ^{*} $P < 0.05$; ^{**} $P < 0.01$; ^{***} $P < 0.001$; NS: non significant as compared to the initial body weight.

OGTT is commonly used to monitor how blood glucose homeostasis is maintained following glucose overload. This test can be applied for the diagnosis of pre-diabetes and diabetes. This test is complementary to glycemia monitoring for diabetes care and could be necessary to detect more subtle changes during the development of insulin resistance [37] [38]. The crude methanol extracts of *A. camperi*, *M. dianthera* and the Polyherb were found to have glucose lowering effects after the oral administration of the extracts in normal rats. This may be due to the presence of hypoglycemic bioactive molecules like flavonoids, terpenoids, alkaloids or saponins that are present in those plants [23].

FBG level was estimated before diabetic induction and after extracts administration. All the three extracts (at doses of 200 and 400 mg/kg per body weight) and standard drug produced significant reduction in the blood glucose level with maximum reduction being achieved with the dose 400 mg/kg for all the extracts showing dose dependent activity. Diabetes is characterized by weight loss and thus was observed in this study. Initially, Alloxan administration brought about marked reduction in body weight of rats. However, the decrease in body weight of the rats was improved by the treatment of the herbal extracts. The extracts with highest activities showed better improvement in body weight compared to the lowest activity extracts.

5. Conclusion

The results from this research suggest that *Aloe camperi*, *Meriandra dianthera* and the Polyherb possess anti-diabetic properties, and therefore can be used as starting point for the development of herbal drugs. However, further studies are required on the isolation and characterization of the bioactive principles responsible for the claimed activities.

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Competing Interests

The authors wish to declare no conflict of interest.

Authors' Contributions

M. S., K.P., K. J., B. G. designed and planned the study; drafted and revised the interpretation and discussion of the results. **M. S. and K. P.** carried out the experimental work and statistical analysis.

All authors read and approved the final manuscript.

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