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ANTIDIABETIC ACTIVITY OF LEAVES AND ROOT EXTRACTS OF KENYAN POPULATIONS OF *DODONAEA VISCOSA* ON STREPTOZOTOCIN (STZ) INDUCED DIABETIC RATS

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ABSTRACT

Diabetes is a metabolic disorder affecting more than 400 million people worldwide. There are three types of diabetes which includes type I *diabetes mellitus* (T1D), type II *diabetes mellitus* (T2D) and gestational diabetes. The disease is associated with polyuria, polydipsia, and glycosuria, and many other underlying conditions. It leads to disability, high financial constraints and ultimately death of the patients. This study therefore intends to seek alternative ways of managing diabetes using herbal remedies. *Dodonaea viscosa* leaves and roots were collected from Baringo, Elgeyo Marakwet, Uasin Gishu and Kwale counties. They were soaked in ethanol and their antidiabetic activity done on male swiss albino rats. Antidiabetic activity was calculated by a glucometer. T-test was used to compare the activity of the roots and leaves. ANOVA was calculated using F-test to find out the activity of various populations. A comparison of activity of Insulin, 200 mg and 400 mg/kg bw extracts was determined using their means. From the results, *D. viscosa* has a strong antidiabetic activity. T-test revealed that there was no significant difference between their activities of roots and leaves as evidenced from $p > 0.05$. F-statistic ($F = 0.545$) indicates that the variances between the populations are not statistically different. The p-value (Sig. = 0.742) further confirms this. Insulin led to the highest decrease in blood sugar, followed by plant extract at 400 mg/kg bw and finally 200 mg/kg bw extracts. More research to isolate pure compounds from this rich plant need to be done.

KEYWORDS: *Dodonaea viscosa*, *Diabetes mellitus*, Antidiabetic activity, Populations.

INTRODUCTION

Diabetes mellitus, commonly referred to as diabetes[1] is a metabolic disorder typified by elevated blood sugar levels brought about by deficiencies in either insulin secretion, insulin action, or both.[2] It is a chronic illness that affects how proteins, lipids, and carbs are metabolized.[3] It is the most prevalent metabolic illness[4] and a public health problem affecting more than 400 million people worldwide.[5,6]

There are three types of diabetes; type I *diabetes mellitus* (T1D), which is dependent on insulin, and type II *diabetes mellitus* (T2D), which is not dependent on insulin[7], as well as gestational diabetes, which affects women who are pregnant.[8] T1D, commonly known as "juvenile diabetes" or ketosis-prone polygenic diabetes, affects children and young adults.[7] T2D is caused by decreased insulin secretion by pancreatic beta cells and poor insulin receptor response.[9] The cause of gestational diabetes which is a temporary condition affecting 2%-10% of expectant women is not known. It is generally believed that insulin resistance can be brought on by weight gain, hormonal imbalances, and chemicals secreted by the placenta that inhibit the effects of insulin.[10]

The disease is characterized by polyuria (the large-scale excretion of diluted urine), polydipsia (the excessive consumption of water), and glycosuria (the large-scale excretion of glucose in the urine.[7] *Diabetes mellitus* is also associated with neuropathy, nephropathy, retinopathy, renal and cardiovascular complications. Insulin replacement therapy is used to treat type I *diabetes mellitus* while oral hypoglycemics are used to treat type II *diabetes mellitus*.[2] Its treatment with allopathic medicine if faced with challenges including the expensive nature of the medications, the disease's progression, and unfavorable side effects.[11] Despite the medication provided, the disease may lead to disability, huge financial constraints and ultimately death of the patient.[12]

The purpose of this study was to evaluate the antidiabetic activity of populations of *Dodonaea viscosa* collected from Baringo, Elgeyo Marakwet, Uasin Gishu and Kwale counties of Kenya using streptozotocin (STZ) induced male rats.

MATERIALS AND METHODS

Study Area

Populations of *Dodonaea viscosa* leaves and roots were collected from Baringo, Elgeyo Marakwet, Uasin Gishu and Kwale counties various regions of the country from 16th may to June 21st 2023. They were transported to the university of Eldoret herbarium for identification by a taxonomist. Some samples were hand-washed in running water, then air-dried for 28 days before ground in readiness for extraction.

Extraction of crude extracts

The protocol developed by[13], with minor modifications was used in extraction. Populations of *D. viscosa* were extracted by maceration with 80% v/v ethanol solution. 500ml of the solvent was added to 100g of the ground plant material (leaves and root barks) in conical flasks and shaken at 200 rpm for a minimum of 24 hours at a temperature of 25 ^{o}C. Following filtration through Whatman No. 1 filter paper, the viscous semisolid masses was dried in a rotary evaporator at 40 °C before being placed in airtight containers for storage at 4 °C.

Breeding of experimental animals

10 male and 18 female Swiss albino mice were bought from the animal house (Kabete campus), university of Nairobi. They were transported to Chiromo campus (University of Nairobi) and placed into 2 cages of 5 males and 9 females in

order to breed. After 7 days the males were separated from the females. On giving birth, adult male mice were fed till they weighed 160-200g for the experiment. Male rats were preferred over the females due to the differences that exist in glucose homeostasis between the different sexes and the effects of the Oestrus cycle which may give varying results.[14] They were then kept in standard environmental settings (24 ± 1 ºC) with 12-hour cycles of light and darkness and given a commercial meal and unlimited access to water. The animals were acclimatized for two weeks before the induction of diabetes.

Induction of diabetes

Streptozotocin (STZ) was administered intraperitoneally to male albino mice in groups 4 after being starved for the previous night in order to cause diabetes. One dose concentration of 6.5 mg/100g of STZ freshly prepared in 1ml distilled water were given to each rat.[15] To confirm stable hyperglycemia, blood samples from the tail vein were taken on days 3 and 7 following injection. Glucose levels will then be calculated using a glucometer (On Call® Plus). Male albino mice with blood glucose levels greater than 11 mmol/L were deemed diabetic.[16,17]

Experimental design

The mice were randomly placed in 5 cages in order to receive different treatments intraperitoneally as shown in Table 1. This was repeated on ethanol extracts of the roots and leaves from different populations.

Table 1: Experimental study design.

Animal group	Status	Treatment	No of mice
I	Normal control	Vehicle only	4
II	Diabetic control	Vehicle only	4
III	Diabetic	Reference drug (Insulin)	4
IV	Diabetic	200 mg/kg bw extract	4
V	Diabetic	400 mg/kg bw extract	4

Oral glucose tolerance test (OGTT)

On day 9, the OGTT was conducted to assess the extract's immediate impact on glucose control. After fasting for the previous night, male albino mice in all groups will receive 2 g/kg of body weight glucose orally. A little amount of blood was drawn from the tail vein before and after the administration of the glucose solution (0, 3, 12 and 24 hours intervals), to determine the impact of the plant extract on blood sugar. The results were represented in mmol/L.

Data Analysis

Antidiabetic activity was calculated by a glucometer (On Call® Plus) and data tabulated in excel spreadsheet to make sorting of data easier. Paired sample t-test was used to compare the activity of the roots and leaves of *D. viscosa*. Analysis of variance (ANOVA) was calculated using F-test to find out the difference between the means and also give the p-value.

RESULTS

Table 2: Antidiabetic activity of *D. viscosa* populations presented as means and standard deviations of 4 males.

		SERGOIT				GAZI				CHEPYOGOT				KABARNET				TURBO				KORIEMA			
		ROOTS		LEAVES		ROOTS		LEAVES		ROOTS		LEAVES		ROOTS		LEAVES		ROOTS		LEAVES		ROOTS		LEAVES	
Treatments	**Duration**	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
Normal Control	0Hr	7.7	1.2	7.7	1.3	7.4	1.1	7.7	0.2	4.9	0.6	7.0	0.8	7.6	0.7	8.0	0.7	5.4	0.4	7.9	0.6	7.6	0.5	7.3	0.3
	3Hr	7.9	0.5	8.0	0.4	7.9	0.5	8.1	0.8	5.3	0.5	6.6	0.2	7.6	0.4	7.9	0.6	5.4	0.5	8.0	0.9	8.5	0.3	7.6	0.7
	6Hr	8.0	0.1	8.0	0.1	8.3	0.4	7.8	0.6	5.3	0.2	7.5	0.9	7.3	0.5	7.4	0.9	5.6	0.4	8.3	1.1	8.3	0.6	7.2	0.5
	12Hr	7.9	0.4	7.8	0.6	7.5	0.5	7.8	0.8	5.4	0.4	7.3	0.8	7.5	0.8	7.6	0.9	5.4	0.7	8.5	0.5	8.5	0.5	7.1	0.7
	24Hr	8.0	0.4	7.9	0.6	7.8	0.3	7.7	0.7	5.3	0.2	7.0	0.6	7.9	1.3	7.9	1.1	5.5	0.3	8.6	0.3	8.9	0.7	7.0	0.4
Diabetic Controls	0Hr	16.7	0.8	18.4	0.8	18.9	1.1	19.8	1.2	19.3	1.3	18.6	1.2	18.8	1.0	19.5	1.7	18.1	2.4	19.8	0.8	18.2	1.8	19.9	1.2
	3Hr	18.3	0.8	19.8	1.3	20.3	0.8	20.9	1.4	20.8	1.2	21.2	0.9	21.7	3.4	20.6	1.8	18.9	2.7	21.0	0.7	19.8	0.9	22.1	0.5
	6Hr	17.1	0.4	17.6	1.6	18.3	1.1	19.0	0.4	19.4	0.3	22.8	2.9	20.9	1.6	19.7	1.0	18.1	2.4	19.7	0.6	19.7	0.3	20.4	1.4
	12Hr	19.4	0.5	19.8	0.9	20.0	0.6	21.1	1.2	21.1	1.4	21.5	0.8	22.2	3.2	20.1	0.4	21.2	4.6	20.6	0.8	19.5	1.1	21.1	1.4
	24Hr	17.6	0.5	18.6	0.7	19.5	0.3	18.3	1.0	18.8	0.8	19.7	1.1	20.4	1.5	17.6	0.8	21.7	2.9	18.2	0.8	19.0	0.9	20.6	1.3
Insulin	0Hr	16.5	0.7	19.0	0.8	15.1	2.6	20.5	2.2	15.2	1.0	19.9	1.3	12.6	1.7	17.4	0.8	18.9	1.0	18.9	1.3	17.9	5.1	17.4	0.9
	3Hr	7.0	0.4	6.3	0.3	7.8	1.5	6.8	0.5	9.0	0.3	6.8	0.5	9.0	1.4	6.6	0.5	9.3	0.5	7.3	0.1	8.5	0.9	7.1	1.1
	6Hr	6.5	0.4	5.7	0.2	6.9	0.5	6.2	0.5	8.5	0.1	6.2	0.6	7.8	0.9	6.2	0.3	8.3	0.4	6.9	0.1	7.3	0.9	6.6	0.6
	12Hr	5.9	0.5	5.1	0.2	5.5	0.5	5.6	0.4	8.2	0.1	5.7	0.3	6.2	0.7	5.8	0.5	8.2	0.5	5.8	0.3	6.1	0.8	5.4	0.5
	24Hr	5.4	0.5	4.4	0.4	5.0	0.3	4.9	0.4	7.9	0.1	5.3	0.3	5.7	0.7	5.5	0.4	7.8	0.4	5.4	0.4	5.3	0.1	5.2	0.2
Extract 200 mg	0Hr	18.2	0.6	16.8	0.7	16.3	1.3	18.8	0.6	16.2	0.9	17.3	0.8	19.5	1.8	19.4	1.3	19.0	1.5	18.2	0.8	17.4	0.7	18.7	0.9
	3Hr	10.8	0.6	7.0	0.2	11.3	1.7	9.2	1.5	22.8	1.3	9.0	1.1	14.4	3.1	13.7	3.9	20.1	1.0	11.5	0.6	12.7	2.5	11.6	2.3
	6Hr	8.4	0.7	6.6	0.2	9.8	0.7	8.5	1.0	13.1	0.5	7.7	1.1	11.5	1.3	10.1	2.6	13.0	1.4	9.2	0.4	10.5	0.5	9.9	1.0
	12Hr	7.3	0.2	5.8	0.5	7.9	0.9	7.1	0.3	11.7	1.2	6.3	0.3	10.5	0.7	8.4	1.2	10.0	0.6	7.7	0.7	8.4	1.5	8.4	1.6
	24Hr	5.7	0.4	5.3	0.4	5.4	0.4	6.1	0.6	8.3	0.9	5.8	0.4	4.8	0.3	6.7	0.6	9.0	1.1	5.1	0.2	5.4	0.4	6.1	0.3
Extract 400 mg	0Hr	18.8	0.8	17.9	1.4	18.1	2.3	17.1	1.3	23.5	2.6	18.3	1.0	19.8	0.9	19.7	0.9	19.8	1.2	18.8	1.0	22.5	2.9	18.1	1.1
	3Hr	7.0	0.2	6.6	0.5	9.7	0.8	6.6	0.6	20.1	10.5	18.3	2.1	8.2	1.4	10.2	0.7	24.1	1.8	9.0	1.9	10.6	0.8	7.3	0.4
	6Hr	6.7	0.2	5.9	0.4	8.7	0.3	6.4	0.6	10.6	0.1	6.8	0.4	7.6	0.8	8.7	0.3	10.3	0.4	7.7	1.0	9.8	0.4	6.8	0.2
	12Hr	6.4	0.3	5.4	0.5	6.5	0.4	6.0	0.7	10.0	0.4	5.5	0.2	5.6	0.9	6.1	0.6	9.0	0.4	5.8	0.5	6.3	0.4	6.4	0.4
	24Hr	5.9	0.3	5.2	0.5	5.5	0.4	5.7	0.8	6.0	0.2	5.4	0.2	4.2	0.2	5.8	0.7	6.1	0.6	5.4	0.4	4.8	0.5	4.9	0.5

A comparison of the activity of roots and leaves of *D. viscosa* from different populations gave the following results as shown in Table 3.

Table 3: Comparison of the activity of roots and leaves.

Population	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	Df	Sig. (2-tailed)
Sergoit Roots & Leaves	.34200	1.24086	.24817	-.17020	.85420	1.378	24	.181
Gazi Roots & Leaves	.06700	1.63546	.32709	-.60808	.74208	.2050	24	.839
Chepyogot Roots & Leaves	1.32700	3.83749	.76750	-.25704	2.91104	1.729	24	.097
Kabarnet Roots & Leaves	.11600	1.64617	.32923	-.56351	.79551	.3520	24	.728
Turbo Roots & Leaves	1.39100	4.01060	.80212	-.26449	3.04649	1.734	24	.096
Koriema Roots & Leaves	.46600	1.61511	.32302	-.20069	1.13269	1.443	24	.162

A comparison of the activity of the different populations was also done to find out the most effective population (Table 4).

Table 4: Comparison of the activity different populations.

Population	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
SERGOIT	50	10.4150	5.48297	.77541	8.8568	11.9732	4.40	19.77
GAZI	50	10.9655	5.68194	.80355	9.3507	12.5803	4.88	21.10
CHEPYOGOT	50	11.9975	6.44692	.91173	10.1653	13.8297	4.88	23.50
KABARNET	50	11.5040	5.87524	.83088	9.8343	13.1737	4.22	22.20
TURBO	50	12.0185	6.10100	.86281	10.2846	13.7524	5.10	24.10
KORIEMA	50	11.4230	5.88139	.83175	9.7515	13.0945	4.83	22.45
Total	300	11.3873	5.89691	.34046	10.7173	12.0572	4.22	24.10

From the above results ANOVA was calculated using F-test to find out the difference between the means and also give the p-value. The results were presented as shown in Table 5.

Table 5: ANOVA result.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	95.447	5	19.089	.545	.742
Within Groups	10301.836	294	35.040		
Total	10397.283	299			

A comparison of the activities of Insulin, 200 mg and 400 mg/kg bw extracts on reduction of blood sugar was also determined. Table 6 shows the results obtained.

Table 6: Comparison of the activities of Insulin, 200 mg and 400 mg/kg bw extracts.

POPULATION		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
SERGOIT ROOTS	Insulin	5	8.2150	4.65684	2.08260	2.4328	13.9972	5.35	16.48
	200 mg/kg	5	10.0600	4.92064	2.20058	3.9502	16.1698	5.65	18.20
	400 mg/kg	5	8.9450	5.50963	2.46398	2.1039	15.7861	5.88	18.77
	Total	15	9.0733	4.73326	1.22212	6.4521	11.6945	5.35	18.77
SERGOIT LEAVES	Insulin	5	8.0600	6.12642	2.73982	.4530	15.6670	4.40	18.95
	200 mg/kg	5	8.3100	4.79298	2.14349	2.3587	14.2613	5.30	16.80
	400 mg/kg	5	8.1700	5.43767	2.43180	1.4182	14.9218	5.15	17.85

	Total	15	8.1800	5.07411	1.31013	5.3700	10.9900	4.40	18.95
GAZI ROOTS	Insulin	5	8.0450	4.11664	1.84102	2.9335	13.1565	4.95	15.13
	200 mg/kg	5	10.1300	4.09197	1.82998	5.0492	15.2108	5.43	16.30
	400 mg/kg	5	9.6650	4.97027	2.22277	3.4936	15.8364	5.53	18.05
	Total	15	9.2800	4.18806	1.08135	6.9607	11.5993	4.95	18.05
GAZI LEAVES	Insulin	5	8.7800	6.59008	2.94717	.5973	16.9627	4.88	20.50
	200 mg/kg	5	9.9200	5.08255	2.27299	3.6092	16.2308	6.05	18.75
	400 mg/kg	5	8.3400	4.92280	2.20154	2.2275	14.4525	5.65	17.13
	Total	15	9.0133	5.21421	1.34630	6.1258	11.9009	4.88	20.50
CHEPYOGOT ROOTS	Insulin	5	9.7700	3.06275	1.36970	5.9671	13.5729	7.93	15.20
	200 mg/kg	5	14.4000	5.48450	2.45274	7.5901	21.2099	8.25	22.78
	400 mg/kg	5	14.0350	7.40715	3.31258	4.8378	23.2322	6.03	23.50
	Total	15	12.7350	5.62882	1.45336	9.6179	15.8521	6.03	23.50
CHEPYOGOT LEAVES	Insulin	5	8.7650	6.26407	2.80138	.9871	16.5429	5.30	19.93
	200 mg/kg	5	9.2200	4.69735	2.10072	3.3875	15.0525	5.83	17.33
	400 mg/kg	5	10.8550	6.80880	3.04499	2.4008	19.3092	5.35	18.30
	Total	15	9.6133	5.62351	1.45199	6.4991	12.7275	5.30	19.93
KABARNET ROOTS	Insulin	5	8.2500	2.75051	1.23007	4.8348	11.6652	5.70	12.58
	200 mg/kg	5	12.1050	5.39555	2.41296	5.4055	18.8045	4.75	19.45
	400 mg/kg	5	9.0900	6.18025	2.76389	1.4162	16.7638	4.22	19.77
	Total	15	9.8150	4.93230	1.27351	7.0836	12.5464	4.22	19.77
KABARNET LEAVES	Insulin	5	8.2750	5.10536	2.28319	1.9359	14.6141	5.45	17.38
	200 mg/kg	5	11.6350	5.05030	2.25856	5.3642	17.9058	6.65	19.40
	400 mg/kg	5	10.0850	5.65289	2.52805	3.0660	17.1040	5.75	19.65
	Total	15	9.9983	5.08767	1.31363	7.1809	12.8158	5.45	19.65
TURBO ROOTS	Insulin	5	10.4850	4.72218	2.11182	4.6216	16.3484	7.78	18.88
	200 mg/kg	5	14.2150	5.06775	2.26637	7.9226	20.5074	9.03	20.08
	400 mg/kg	5	13.8600	7.69051	3.43930	4.3110	23.4090	6.13	24.10
	Total	15	12.8533	5.79952	1.49743	9.6417	16.0650	6.13	24.10
TURBO LEAVES	Insulin	5	8.8500	5.66985	2.53563	1.8100	15.8900	5.40	18.90
	200 mg/kg	5	10.3300	4.95339	2.21522	4.1796	16.4804	5.10	18.15
	400 mg/kg	5	9.3400	5.47459	2.44831	2.5424	16.1376	5.38	18.77
	Total	15	9.5067	5.01641	1.29523	6.7287	12.2847	5.10	18.90
KORIEMA ROOTS	Insulin	5	9.0100	5.09836	2.28006	2.6795	15.3405	5.30	17.88
	200 mg/kg	5	10.8900	4.55452	2.03684	5.2348	16.5452	5.35	17.43
	400 mg/kg	5	10.8000	6.93776	3.10266	2.1856	19.4144	4.83	22.45
	Total	15	10.2333	5.28287	1.36403	7.3078	13.1589	4.83	22.45
KORIEMA LEAVES	Insulin	5	8.3200	5.13941	2.29842	1.9386	14.7014	5.15	17.40
	200 mg/kg	5	10.9300	4.80193	2.14749	4.9676	16.8924	6.10	18.73
	400 mg/kg	5	8.6700	5.32134	2.37978	2.0627	15.2773	4.85	18.05
	Total	15	9.3067	4.86405	1.25589	6.6130	12.0003	4.85	18.73

DISCUSSION

For the entire study, male rats were preferred over the females as they are more responsive to STZ.[18] Oestrogens produced in females' Oestrus cycle also partly contributes to protection against diabetes.[19,20] The rats in group I (normal control) had their blood sugar level low (below 11mmol/L) throughout the study period. Those in group II (Diabetic control) remained diabetic (above 11mmol/L) due to the effect of Streptozotocin (STZ) injection. The diabetic rats in group III were treated with insulin hormone making their blood sugar levels significantly reduced to normal. The diabetic rats in groups IV and V were treated with *Dodonaea viscosa* crude extract of leaves and roots at 200mg/kg bw and 400mg/kg bw respectively. The rats in these two groups also registered a drop in their blood sugar levels to normal from the 3rd to the 24th hour.

A comparison of the activity of roots and leaves done using paired sample t-test revealed that there was no significant difference between their activities. This was evident from the level of significance which was >0.05. This could be attributed to the activity of secondary metabolites synthesized by the leaves of plants and subsequent transportation to other organs including the stem.[21,22]

From table 5, the F-statistic (F = 0.545) indicates that the variances between the groups are not statistically different from each other. The p-value (Sig. = 0.742) further confirms this, as it is greater than the commonly used significance level of α = 0.05. Based on this ANOVA test, there is no enough evidence to conclude that the means of the groups/populations are statistically different.

For all the populations, insulin hormone led to the highest reduction in blood sugar level bringing the blood sugar to the norm. This was followed by the plant extract at 400 mg/kg bw and finally 200 mg/kg bw extracts. Most populations brought about a significant reduction in blood sugar levels except a few populations which were not able to reduce blood sugar below 11mmol/L. These included Turbo roots at 200 mg/kg bw, Kabarnet leaves at 200 mg/kg bw, Kabarnet roots at 200 mg/kg bw and Chepyogot roots at both 200 mg/kg bw and 400 mg/kg bw.

D. viscosa crude extract generally led to a significant reduction in the level of blood sugar from the rats under study. This could be attributed to the combined action of various compounds, including flavonoids, saponins, di- and triterpenes, as well as a mixture of phenolics found in the plant.[23,24] Biochemical evaluations on *D. viscosa* revealed that the plant has a variety of components such as oils and fats, cyclic and acyclic phenolics, tricyclic flavonoids, steroidal compounds, reducing sugar, cardiac glycosides, and carbohydrates which may also be responsible for this activity.[25]

CONCLUSION

D. viscosa crude extract has a potential to reduce blood sugar in Swiss albino rats. The activities of the roots, leaves and the different populations were not significantly different.

RECOMMENDATION

We recommend isolation of pure compound to be done on this plant to find out the compound responsible for this antidiabetic activity.

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