Juniper Publishers
key to the Researchers

ANTIDIABETIC PROPERTIES OF BROWNEA COCCINEA, "ROSE OF THE MOUNTAIN" EXTRACTS ON ALLOXAN INDUCED DIABETIC RABBITS

CURRENT RESEARCH IN DIABETES & OBESITY JOURNAL

Antidiabetic Properties of *Brownea Coccinea*, "Rose of the Mountain" Extracts on Alloxan Induced Diabetic Rabbits

Jagessar RC[1]*, Ramnanan R[1], Persaud D[1], Rasul BS[1] and Gopie S[2]

[1]Department of Chemistry, University of Guyana, Guyana

[2]Department of Pharmacy, University of Guyana, Guyana

*Corresponding Author

Jagessar RC, Department of Chemistry, Faculty of Natural Sciences, University of Guyana, Guyana

Published By

Juniper publishers Inc.

United States

August 31st, 2020

Abbreviations

TC: Total Cholesterol; TG: Triglycerides; HDL-CP: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; MDA: Malondialdehyde; Co-Q10: Coenzyme

List of Contents

Abstract

The hypoglycemic effect of the aqueous extract of *Brownea coccinea*, "Rose of the Mountain" on three different groups of Alloxan induced diabetic rabbits and a control group were investigated over a 21days period. The target rabbits' groups were subjected to blood glucose and body weight analyses. It was found that for all the groups, there was an overall hypoglycemic effect. However, the various treatments induced hypoglycemic effect to varying percentage. For the glibenclamide treated group, there was a 20.59 % reduction in blood glucose level as compared with a 12.42% reduction in blood glucose level, using the aqueous extract. The hypoglycemic effect followed the trend: glibenclamide treated groups > control group > aqueous extract treated group > feed only group. Body weight is an indicator of obesity and diabetes. Overall, the body weight increased, and this was variable for the various treatments. Glibenclamide induced a 40.38% increase in body weight, whereas the aqueous extract, resulted in an increase in body weight of 64.58%. The increased in body weight followed the trend: control group > feed only group > aqueous extract treated group > glibenclamide. Anova analyses were done to investigate whether there are significant differences between the various groups per treatment and between members of the same group. In most instances the P value was found to be greater than 0.05, indicating that there weren't any significant differences between treatments per group and members of the same group. This was supported by F value < F critical, over 90% of results statistically analysed. Thus, *Brownea coccinea,* "Rose of the Mountain" can be regarded as an hypoglycemic plant and should be added to the list of plants with hypoglycemic effects.

Introduction

Diabetes mellitus is a chronic health problem, with long term consequences that are potentially preventable. It is a non- communicable disease worldwide. It is a heterogeneous group of disease, characterized by high blood glucose levels, resulting from impaired insulin secretion, impaired insulin action, or both [1-4]. Not only is it an incurable lifelong disease, it is also a risk factor for cardiovascular diseases. There are three major types of diabetes. Type 1 diabetes which leads to absolute insulin deficiency due to autoimmune β-cell destruction, type 2 diabetes which occurs when there is insulin resistance, due to a progressive loss of β-cells and Gestational Diabetes Mellitus (GDM) which is diagnosed in the second or third trimester of pregnancy. Type 1 diabetes and type 2 diabetes are heterogeneous diseases in which clinical presentation and disease progression may vary considerably. However, for both type 1 and type 2 diabetes, various genetic and environmental factors can result in the progressive loss of β-cell mass and/or function that manifests clinically as hyperglycemia. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide. Due to the fact that diabetes is a lifelong disease, where treatment is prolonging, detrimental side effect and target organ damage is evident. The major concern in this study is Type 2 diabetes which plagues the Guyanese population. This type of diabetes is primarily associated with insulin secretory defects related to inflammation and metabolic stress amongst other contributors, including genetic factors. This disease also has been found to result in oxidative stress and impaired antioxidant defense system. This causes alteration in antioxidant enzymes, due to impaired lipid peroxidation and impaired glutathione metabolism [1-3]. It is associated with the deterioration and loss of proper functioning of the β-cells of islet of Langerhans of the pancreas and as a result of receptor malfunctioning. Thus, glucose is not taken up by the tissues and the glucose level in the blood increases causing hyperglycemia which is a major symptom of diabetes mellitus. As such, prolonged and more severe hyperglycemia contributes to other pathological conditions associated with diabetes mellitus. In addition, other factors such as oxidative stress damages insulin-producing cells of the pancreas which is also a determining factor for the progression of diabetes. In a hyperglycemic state, the body tries to remove excess glucose by excreting in the urine. This increases urine output, causing glycosuria and result in frequent thirst. In addition, the body is deprived of glucose energy and seeks alternative energy sources such as fats and muscle tissues, leading to weight loss[4]. A diminishing growth effect and increased predisposition to certain infections, may also be present with chronic hyperglycemia [5]. These combinations along with polyuria, polydipsia, polyphagia, and blurry vision produces the common symptoms of diabetes [6]. As this disease progresses, vascular damage ensues leading to severe diabetic microvascular and macrovascular complications [7]. Therefore, diabetes covers a wide range of diseases which are the major causes of chronic morbidity and death in diabetic subjects [8]. Being described as the "the perfect epidemic" this condition affects an estimated 387 million people worldwide. The World Health Organisation, WHO, reported that approximately 150 million people have diabetes mellitus worldwide, and that this number may well double by the year 2025. According to the WHO, the incidence of diabetes has risen dramatically over the past years with a current prevalence of 9% and it is expected to affect more than 500 million adults by 2030. North America and the Caribbean are the regions with a higher prevalence of 11%, having 37 million people affected. In 2012, an estimated 1.5 million deaths were directly caused by diabetes, with 80% deaths occurring in low and middle-income countries [9]. A national survey revealed the total number of diagnosed diabetic cases in Guyana as 49,800, with an estimated number of 1025 deaths: a substantially high value for a population as small as that of Guyana's [10].

The World Health Organization, categorized Guyana, South America, as a lower middle-income country with a population of 767,000 people, 9.1% of which have been documented cases of diabetes which is a risk factor for leading 33% of documented cardiovascular cases. WHO has reported an ethnic link, where Indo-Guyanese accounts for (43%) diabetic patients, Afro-Guyanese (30%) and Amerindian (9%). Diabetes is one of the leading non-communicable diseases in Guyana which has high mortality and morbidity [15]. Although, antidiabetic agents such as insulin, biguanides, thiazolidinediones and a glucosidase inhibitor are available in Guyana to treat diabetes, a safe and effective treatment paradigm is yet to be achieved [11]. This is due to that fact that these drugs fail to significantly reduce the course of diabetic complications and have limited use, because of their undesirable pathological conditions and high secondary failure rates. Therefore, it is essential to discover more effective antidiabetic agents with few adverse effects, low costs and ease of accessibility [12]. In recent years, there has been a resurgence of interest in medicinal plants for the treatment of diseases [12]. A World Health Organization (WHO) study shows that 80% of the world's population solely relies on medicinal plants for their primary health care needs [13]. Medicinal plant extracts, having antidiabetic properties can be a useful source for the development of oral hypoglycemic agents in both animal models and human subjects [14]. Over 350 plants are used in the treatment of *diabetes mellitus*, but only a small number of these plants had gained scientific and medical evaluation to assess their effectiveness and efficacy. For the management of diabetes, the World Health Organization [15]. (WHO) has recommended the evaluation of traditional plant treatments as they are effective, non-toxic, with little or no side effects and are considered to be excellent candidates for oral therapy [16].

Synthetic drugs currently in use for diabetic treatment include sulfonylureas (such as glibenclamide). Glibenclamide stimulate the release of insulin from β-cells of the pancreas via the mechanism of action responsible for the inhibition of the potassium (K^+) channels, causing the calcium ion (Ca^{2+}) induced depolarization of the cells which in turn permit the release of insulin. However, these antidiabetic medications have undesirable side effects, including weight gain, hypoglycemia, nausea and diarrhea. These contributes a great deal to non-compliance in patients which can lead to further deleterious progression of their condition and result inevitably, in the increased mortality rate of the disease. The first line conventional therapies for type 2 diabetes include biguanides (metformin) and sulfonylureas (glyburide). Insulin sensitivity is said to increase with metformin while the role of the sulfonylureas is increasing insulin secretion. Other anti-diabetic agents include alphaglycosidase inhibitors, thiazolidinedione, metiglidine analogues and drugs that affect the incretin system. Acting on the gastrointestinal tract to decrease glucose absorption are the alpha glucosidase inhibitors, while thiazolidinediones improve insulin sensitivity by affecting multiple intracellular metabolic pathways and the Metiglidine increase insulin secretion. These drugs can be used in the form of monotherapy or in combination [17].

Over time, antidiabetic medications, especially oral anti-diabetic medication used to treat type II diabetes have shown to have decreased efficacy, detrimental side effects such as hypoglycemia, weight gain, increase risk of heart attack, increase susceptibility to genital and urinary infections, pharyngitis, increase risk of bladder cancer, in some cases organs damage. Patients who are in poverty face financial constrains to treat their diabetes, which results in poor patient compliance and uncontrolled diabetes. The objectives of the research were: To identify the potential antidiabetic properties of *Brownea coccinea*, “Rose of the Mountain” bark extract in lowering blood glucose levels in alloxan induced diabetic rabbits; To measure the hypoglycemic effect of mentioned aqueous plant extract on blood glucose levels in alloxan induced diabetic rabbits.**;** To compare the hypoglycemic properties of the mentioned aqueous plant extract with that of glibenclamide from blood glucose levels of alloxan induced diabetic rabbits; To tract the body weight of the rabbits at the initiation and at the end of the experiment. *Brownea coccinea* is a species of small native evergreen tree with compound leaves and clusters of bright scarlet flowers in the subfamily *Caesalpinioideae* of the family *Fabaceae*. Common names include scarlet flame bean, mountain rose and cooper hoop. The species is native to Colombia, Ecuador and Guyana [18].

In Trinidad, infusions of the flowers are used as a remedy for colds and coughs. The fresh bark of the tree is used as an anti-hemorrhagic and applied to wounds. Other uses for the plant are to stimulate blood flow to the pelvic area and uterus in order to stimulate menstruation, and as an abortifacient. It is commonly used to treat gynecological disorders such as dysmennorrhea and menhorrhagia [19]. *Brownea coccinea*, (Fabaceae) is a small to mid-sized tree with slender branches and a rounded crown reaching an height of about 12ft. They have compound leaves which are 10-35cm. long, containing 4-10 leaflets. Leaflets are oblong or elliptic, pointed at the apex and 4-23cm. long and 1.5-6.5cm. wide and smooth. Flowers are tubular with orange-scarlet calyx and petals and 10-12cm, protruding stamens in heads 7-9cm. wide, surrounded by downy red bracts and in clusters of 2-3 on branches are trunks. Seed pods are brown and 12-24cm. long, 4cm. wide and contain 4-10 flat seeds [20] (Figure 1).

Figure 1: Rose of the mountain, inflorescences.

This plant is found in the Iwokrama Rainforest of Guyana and the hinterland region. There is little recorded information on this plant, but it is locally used (bark) in Guyana as a traditional medicine to treat diabetes and lower cholesterol. However, there were no studies found in the literaure, which confirm findings of hypoglycemic and antidiabetic properties of this plant.

Some of the research questions that were asked during the research were.

Will *Brownea coccinea*. ("Rose of the mountain") bark extract has hypoglycemic properties to lower blood glucose levels in the alloxan induced diabetic rabbits?

a) **Alternative Hypothesis (H1):** *Brownea coccinea* ("Rose of the mountain") bark extract used will provide a positive result in lowering blood glucose levels in alloxan induced rabbits as compared to the glibenclamide .

b) **Null Hypothesis ($H_0$1):** *Brownea coccinea* ("Rose of the mountain") bark extract will not effectively lower blood glucose levels in the alloxan induced rabbits as compared to the glibenclamide.

How well would the rabbits tolerate the plant extracts over the glibenclamide?

a) **Alternative Hypothesis (H2):** The rabbits will better tolerate the plant extract as compared to the glibenclamide.

b) **Null Hypothesis ($H_0$2):** The rabbits will not tolerate the plant extract as compared to the glibenclamide.

Will there be variation in body weight in the alloxan induced rabbits after the consumption of the plant extraction?

a) **Alternative Hypothesis (H3):** An apparent increase of body weight will be observed after the administration of the plant extracts in the alloxan induced diabetic rabbits.

b) **Null Hypothesis ($H_0$3):** There is no apparent increase in weight noted after administration of the extracts in alloxan induced rabbits.

c) **Alternative Hypothesis (H3):** An apparent increase of body weight will be observed after the administration of the plant extracts in the alloxan induced diabetic rabbits.

d) **Null Hypothesis ($H_0$3):** There is no apparent increase in weight noted after administration of the extracts in alloxan induced rabbits.

Plants have been used over the years for their antimicrobial [21-28], anticancer [29-34], antioxidant [35-43] and antidiabetic activities [35-43]. It was mankind first source of medication and is still. However, there is a still a need to expand the antidiabetic profile of plants/plant extracts with a view to produce selective potent medication with little or no side effects. To this end, this paper reports the use of "Rose of the mountain", *Brownea coccinea*, bark extract to lower blood glucose concentrations in alloxan induced diabetic rabbits, with a view to further develop antidiabetic herbal medications. However, to date, there are no studies found in the literature, which confirm findings of hypoglycemic and antidiabetic properties of this plant. Rabbits were selected for conducting this research because they have many biological similarities to humans and they are also mammals. They share common lipid metabolism, cholesterol metabolism and transport with humans.

There are various types of phyto chemical constituents present in plant material, acting singly or in combination that may be responsible for antidiabetic activities. Alkaloids inhibit alpha-glycosidase which decrease glucose transport through the intestinal epithelium. Flavonoids suppress the glucose level, reduce plasma cholesterol and triglycerides. Flavonoids increase hepatic glucokinase activity by enhancing the insulin release from pancreatic islets. Imidazoline compounds stimulates insulin secretion in a glucose dependent level. Saponins stimulates the release of insulin and blocks the formation of glucose in the bloodstream [32-42].

The antidiabetic activities of a range of plant extracts have been reported [34-42]. However, to date, there is no written documentation of the antidiabetic activity of "Rose of the Mountain" (Fabaceae). Thus, the antidiabetic activies of other members of the Fabaceae were investigated. *Prosopis farcta* (Fabaceae) has been used as a traditional herbal medicine for treating diabetes *mellitus*. The antidiabetic mechanisms of infusion (INF) extract of *P. farcta* and discovering the active extract for the first time has been reported [44]. Antihyperglycemic, hypoglycemic and cytotoxic activities of methanolic extract of *Albizia lebbeck* and *Trigonella corniculata* were studied. Hypoglycemic action of plants was investigated using normoglycemic rabbits (acute study only), whereas antihyperglycemic activity was studied using alloxan induced diabetic rabbits (acute and chronic study). Results revealed both plant extracts and their mixture having significant anti diabetic potential at dose of 200mg/kg comparable to standard drug glibenclamide (0.5mg/kg). Brine shrimp assay was used for the determination of cytotoxicity and results showed plants to have low toxic potential (LD50 > 1000 microgram). The study conducted proves both plants and their mixture to have high potential for the treatment of diabetes [45].

The antidiabetic activities of *Acacia arabica* (*Fabaceae*) plant extracts on albino rats have been reported [46]. Thirty-six female albino rats were divided into six equal groups; control, streptozotocin-induced diabetic group, untreated and two groups treated with *Acacia arabica* extract orally for 21 days (100mg/kg for the second group and 200mg/kg for the third group). After twenty one (21) days, the serum glucose, insulin, Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Malondialdehyde (MDA), and Coenzyme Q10 (Co-Q10) were tested. A significant decrease in levels of serum glucose, insulin resistance, TC, TG, LDL-C, MDA and a significant increase in HDL-C and Co-Q10 was observed in the treated diabetic groups when compared to the untreated diabetic group. The changes were dose dependent. The results indicate that *Acacia Arabica* extract has hypoglycemic, hypolipidemic, and antioxidant properties, therefore, it can be investigated for its efficacy in the treatment of diabetes in humans [46].

The methanolic water extracts of the aerial parts of four *Onobrychis* species, *O. albiflora O. argyrea Boiss, O. galegifolia* Boiss., and *O. tournefortii* (Willd.) Desv. were tested for their antidiabetic activities on alloxan-induced diabetic mice. The highest activity was observed with treatment of *O. albiflora* aerial part extract. Significant decrements were detected in the blood glucose levels as follows: (180.83±47.48 mg/dL) and (252.83±50.47mg/dL) at 100mg/kg and 200mg/kg doses of *O. albiflora*, respectively, when compared to the isotonic saline solution control group, eliciting a blood glucose level of 494.20±27.32. Among the tested standard compounds, rutin and isoquercetin were detected in the examined species. The highest amount of rutin (1.1981±0.001) and isoquercetin (0.7318±0.0197) were found in *O. albiflora* and *O. argyrea* respectively. Antidiabetic activities of the tested *Onobrychis* species seem to indicate a possible correlation with their rutin and isoquercetin contents. Thus, rutin and isoquercetin may be the antidiabetic compounds that contribute to the antidiabetic activity of the tested *Onobrychis* species [47].

Research Methodology

Research Design

This experiment featured a fixed effects model experimental design.

Methodology

Fresh, brown bark from of the *Brownea coccinea. ("*Rose of the mountain") was collected, washed, dried and identified. The bark was then weighed and cut into pieces and extracted once with distilled, deionized water, using heat. The weight of the bark was kept constant in all experiments undertaken. A total of twelve (12) rabbits were used in the experiment i.e. three per cage. It was ensured that the cages were well cleaned and disinfected, prior to the conduct of the experiment. All animals were tagged. Rabbits were colour tagged for identification, the control group (Group 4) were normal domesticated rabbits, approximately five (5) to six (6) months old. The test subjects that were induced with diabetes, using alloxan, were all albino rabbits, approximately five to six months old as well. Prior to the start of the experiment, blood samples were taken from each rabbit and the blood glucose level read using the glucometer. All rabbits were divided into four groups: Group A, Group B, Group C and Group D.

Group A, Group B and Group C consisted of rabbits, made diabetic using Alloxan monohydrate. Group D consisted of rabbits that were not induced diabetic but that were normal. Diabetic groups (A, B and C) were used 48 hours after administration of Alloxan monohydrate in saline. This was indicated by glucose level greater than 140mg/dl using a glucometer. Alloxan monohydrate was solubilized with 0.2ml saline (154 mM, NaCl), just prior to the injection. It was also administered according to the rabbit's weight, 150mg/kg. Group A represented rabbits that were diabetic (140 mg/dl) and received nothing else, other than normal feed. Group B represented rabbits that were diabetic (140mg/dl) and was administered with a known amount of glybenclamide (hypoglycemic agent). Group C represented rabbits that were diabetic and administered with a definite volume of the aqueous extract. The aqueous extract was administered at 42mg/kg per day. All groups were fed normal feed for the period of three weeks.

The experiment proceeded for a period of three weeks. Blood samples were taken twice a week (day 1, day 4, day 7, day 10, day, 14, day 17 and day 21) and read with the glucometer i.e. six readings in triplicates at the completion of the experiment. At the completion of the experiment, blood samples were again be analyzed for cholesterol, serum triglycerides etc.

Variables

Independent variables: *Brownea coccinea*. ("Rose of the Mountain") Glibenclamide

Dependent variable: Body weight, Blood glucose concentration

Population

The targeted population were rabbits which were obtained from Mandela Avenue, Georgetown. The subjects consisted of nine (9) males and three (3) females, with varying body weight

Sample

The method of random selection were used to accommodate the nine (9) males and three (3) females on a farm located at Mandela Avenue, Georgetown.

Experimental treatment

The aqueous plant extract was used in the conduction of this experiment: *Brownea coccinea*. (Rose of the Mountain) bark extract. Plant collection and extraction: “Rose of the mountain” bark was collected, washed and dried and subsequently ground and boiled in water for thirty minutes. After cooling, it was filtered and the extract was kept refrigerated, until necessary for administration to alloxan induced diabetic rabbits.

Housing of animals and diet

The UCSD housing requirements for laboratory animals and the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes (December 2014), was followed to ensure the animals had enough space and not subjected to environmental stress which can affect their well-being and as well as the results of the experiments. The cage sizes were 24.5x30.5 x 48.28cm, made out of wood and mesh, there were three rabbits allotted per cage. The animals were kept in normal night and day cycles exposed to temperature 23-28^{0}C. The cage pans were cleaned daily. The food fed to the rabbits was maintained as followed by the Standard Diets Laboratory Animal Diets (2016-2018), which included giving the animals pellets fortifies with vitamins and minerals. Vitamin C and foliage, tanner grass (*Brachiaria arrecta)* was also supplied to their diet to maintain immunity and filing of their teeth, with constant supply of distilled water.

Blood glucose concentration test

This was done using a glucometer: Control D. The rabbits were tested every 3 days by using a lancer to prick the tip of their tails.

Statistical analysis: The results were recorded as mean and standard deviation. All data analyses were done using 1- way Analysis of Variance (ANOVA). A P value or significant value of < 0.05 will be considered significant. Also, the calculated F value must be less than F critical.

Validity: Validity: the validity of the study was very clear as the methodology was guided from similar research with minor modifications. The compilation of the methodology and content were both guided by Professor Raymond Jagessar, which ensure the validity of the research.

Reliability: to ensure consistency of results obtained, three (3) rabbits were placed in different cages and separated from each other during the experiment.

Results

(Table 1 & 2, Figures 2-4)

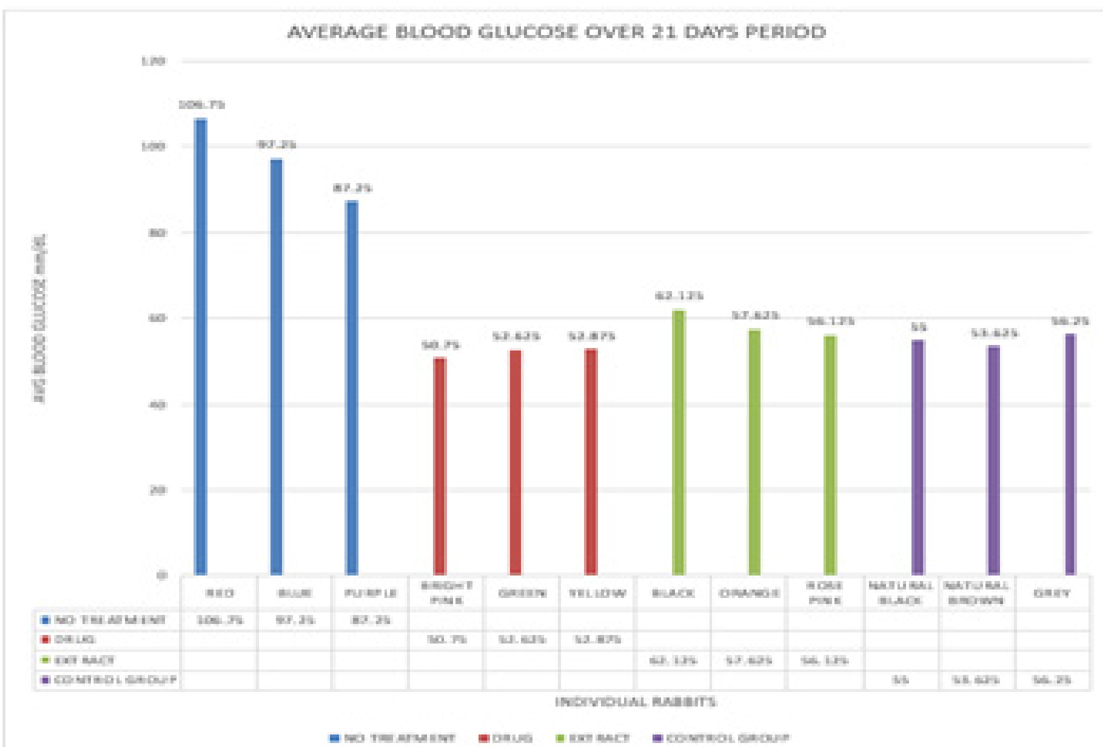

Figure 2: A plot of average blood glucose (mm/dL) verses various treatments on rabbit group.

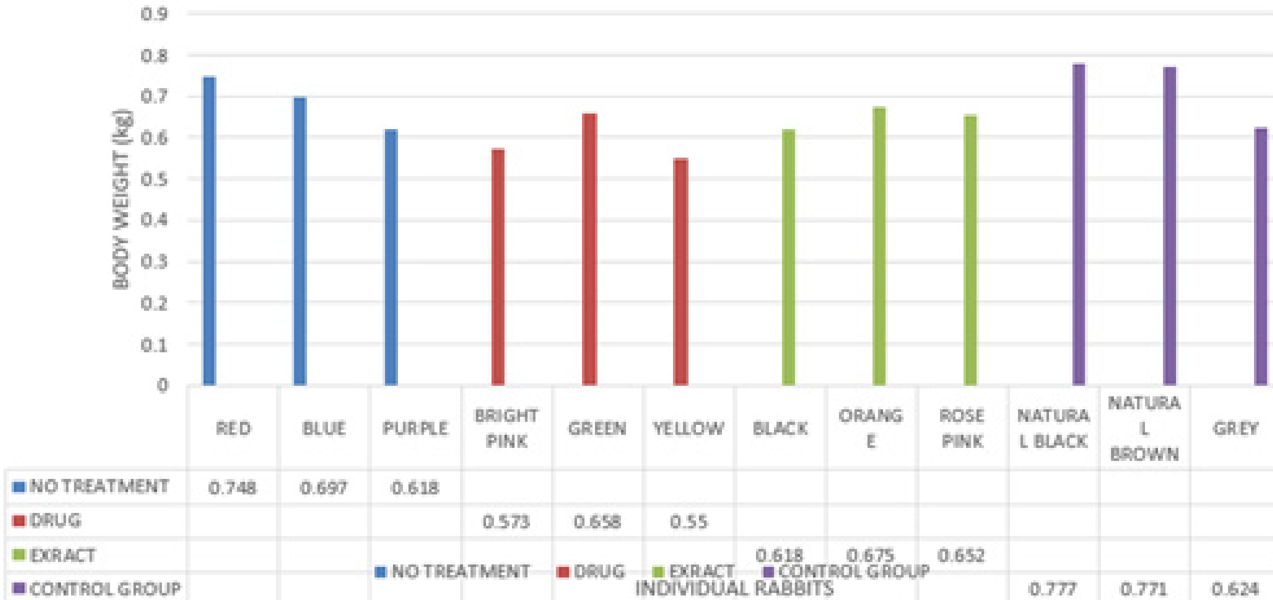

Figure 3: A plot of body weight versus various treatments on rabbit group.

Figure 3: Correlation of average body weight with blood glucose levels.

Statistical Analysis Using One Way Anova

Blood Glucose comparison using ANOVA

(Table 3-5)

Table 1: Showing the days which, the blood glucose of the rabbits were extracted and tested in (mg/dl).

Test Subjects, Colour Coded	Gender	16-May-19	19-May-19	22 -May-19	25-May-19	28-May-19	31-May-19	3-Jun-19	6-Jun-19	Average Blood Glucose (mg/dl)
Group 1- feed only										
Red	M	139	106	115	110	99	90	100	95	106.75
Blue	F	130	98	95	90	90	105	90	80	97.25
Purple	F	128	90	95	85	82	73	80	65	87.25
Average		132.33 ± 5.85	98 ±8.0	101.67 ±11.54	95±13.23	90.33± 8.50	89.33±16.01	90 ±10.0	80±15	
Group2 - Glibenclamide										
Bright pink	M	60	55	52	55	42	50	40	52	50.75
Green	M	54	42	60	57	50	53	50	55	52.63
Yellow	M	56	40	58	60	47	47	45	70	52.88
Average		56.67±3.05	45.67±8.14	56.67±4.16	57.33±2.52	46.33±4.041	50±3.0	45 ±5.0	59±9.64	
Group3 -aqueous extract										
Black	M	63	72	66	65	55	60	56	60	62.13
Orange	M	59	45	68	70	57	57	55	50	57.63
Rose pink	M	55	53	50	68	48	67	63	45	56.13
Average		59±4.0	56.67 ±13.86	61.33 ±9.865	67.67±2.51	53.33± 4.73	61.33±5.13	58±4.36	51.67±7.64	
Group4 – control										
Natural Black	F	65	55	43	53	55	58	56	55	55
Natural brown	M	50	52	45	50	65	55	50	62	53.63
Natural green	M	60	55	55	65	58	60	45	52	56.25
Average		58.33±	54.4±1.73	47.67±6.429	56 ±6.429	59 ±6.429	57.67 ±2.52	50.33±5.50	56.33±5.13	

Table 2: Showing the days which the weight of the rabbits were measured in (Kg).

Test subjects, colour coded	Gender	16-May-19	19-May-19	19-May-19	25-May-19	28-May-19	31-May-19	3-Jun-19	6-Jun-19	Average weight (Kg)
Group 1- feed only										
Red	M	0.5	0.73	0.68	0.73	0.73	0.73	0.95	0.95	0.75
Blue	F	0.45	0.54	0.68	0.73	0.77	0.77	0.82	0.82	0.7
Purple	F	0.45	0.68	0.59	0.59	0.59	0.59	0.73	0.73	0.62
		0.47 ± 0.029	0.65±0.080	0.65±0.052	0.68±0.052	0.69±0.095	0.69±0.09	0.83±0.110	0.83±0.11	
Group2 - Glibenclamide										
Bright pink	M	0.5	0.45	0.63	0.54	0.54	0.54	0.68	0.68	0.57
Green	M	0.63	0.5	0.59	0.63	0.63	0.63	0.82	0.82	0.66
Yellow	M	0.45	0.45	0.5	0.54	0.54	0.54	0.68	0.68	0.55
Average		0.52±0.093	0.466 ±0.029	0.57± 0.066	0.57±0.051	0.57±0.051	0.57±0.051	0.73±0.066	0.73±0.066	

Group3 -aqueous extract										
Black	M	0.45	0.59	0.59	0.59	0.63	0.63	0.73	0.73	0.62
Orange	M	0.54	0.63	0.68	0.63	0.63	0.63	0.82	0.82	0.67
Rose pink	M	0.45	0.63	0.59	0.63	0.63	0.63	0.82	0.82	0.65
Average		0.48 ±0.052	0.62± 0.025	0.62 ± 0.051	0.62±0.023	0.63±0.000	0.63±0.000	0.79±0.052	0.79±0.052	
Group4 - control										
Natural Black	F	0.73	0.73	0.73	0.73	0.73	0.73	0.95	0.91	0.78
Natural brown	M	0.63	0.73	0.68	0.73	0.73	0.73	1	0.95	0.77
Natural green	M	0.5	0.59	0.59	0.59	0.59	0.59	0.77	0.77	0.62
Average		0.62 ± 0.115	0.68±0.0808	0.67±0.079	0.68±0.080	0.68 ±0.080	0.68±0.080	0.91±0.120	0.87±0.095	

Table 3: Treatment with aq. Extract: blood glucose comparison.

Days		F	P value
Day_0	Between Groups	.018	0.900
Day-3	Between Groups	0.109	0.758
Day-6		4.041	0.115
Day-9	Between Groups	5.889	0.072
Day-12	Between Groups	2.219	0.211
Day-15	Between Groups	1.235	0.329
Day_18	Between Groups	3.574	0.132
Day-21	Between Groups	0.772	0.429

Table 4: Treatment with glipalamide: blood glucose comparison.

Days		F	P value
Day-0	Between groups	0.123	0.743
Day-3	Between groups	3.005	0.158
Day-6	Between groups	4.142	0.112
Day-9	Between groups	0.077	0.795
Day-12	Between groups	11.883	0.026
Day-15	Between groups	11.50	0.028
Day-18	Between groups	1.542	0.282
Day-21	Between groups	0.179	0.694

Table 5: Treatment with feed only: blood glucose comparison.

Days		F	P value
Day-0	Between groups	177.281	0.00
Day-3	Between groups	86.687	0.001
Day-6	Between groups	50.084	0.002
Day-9	Between groups	19.172	0.012
Day-12	Between groups	29.220	0.006
Day-15	Between group	11.453	0.028
Day-18	Between groups	36.217	0.004

Weight comparison with Anova

(Table 6-8)

Table 6: Treatment with feed only: Weight comparison.

Days		F	P value
Day -0	Between groups	4.991	0.89
Day-3	Between groups	0.205	0.674
Day-6	Between groups	0.108	0.759
Day-9	Between groups	0.000	1.00
Day-12	Between groups	0.34	0.862
Day-15	Between groups	0.34	0.862
Day-18	Between groups	0.600	0.482
Day -21	Between groups	0.266	0.633

Table 7: Treatment with Glibenclamide: weigh comparison.

Days		F	P value
Day-0	Between groups	1.191	0.336
Day-3	Between groups	19.118	0.012
Day-6	Between groups	2.761	0.172
Day-9	Between groups	4.173	0.111
Day-12	Between groups	4.173	0.111
Day-15	Between groups	4.173	0.111
Day-18	Between groups	4.592	0.099
Day-21	Between groups	4.364	0.105

Table 8: Treatment with aqueous extract: weight comparison.

Days		F	P value
Day-0	Between groups	3.675	0.128
Day-3	Between groups	1.887	0.242
Day-6	Between groups	0.845	0.410
Day-9	Between groups	1.887	0.242
Day-12	Between groups	1.306	0.317
Day-15	Between groups	1.306	0.317
Day-18	Between groups	2.356	0.200
Day-21	Between groups	1.937	0.236

Overall Anova analyses

(Table 9-16)

Table 9: Glibenclamide.

Source of Variation	*F*	*P-value*	*F crit*
Between Groups	2.778812	0.08498	3.4668
Within Groups			

Table 10: Group 3: Aqueous extract.

Source of Variation	*F*	*P-value*	*F crit*
Between groups	0.188501	0.829586	3.4668
Within groups			

Table 11: Group 4: Control group

Source of Variation	*F*	*P-value*	*F crit*
Between Groups	1.308699	0.291317	3.4668
Within Groups			

Table 12: Control Group Statistical Analysis for Body Weight Measurement: Group 1: Feed only.

Source of Variation	*F*	*P-value*	*F crit*
Between Groups	0.348369	0.709841	3.4668
Within Groups			

Table 13: Group 1: Feed only

Source of Variation	*F*	*P-value*	*F crit*
Between Groups	2.180062	0.137947	3.4668
Within Groups			

Table 14: Group 2: Glibenclamide

Source of Variation	*F*	*P-value*	*F crit*
Between Groups	2.902204	0.077115	3.4668
Within Groups			

Table 15: Group 3: aqueous extract.

Source of Variation	*F*	*P-value*	*F crit*
Between Groups	0.635603	0.539502	3.4668
Within Groups			

Table 16: Group 4: Control group.

Source of Variation	*F*	*P-value*	*F crit*
Between Groups	5.110073	0.01555	3.4668
Within Groups			

Discussion

Fasting Blood Glucose (FBG) levels of rabbits and the baseline body weight were tested over a 21 days period. During this experimental interval, there were no signs of intoxications, restraint of animals, chills, hesitation, rustling hairs, anuria and finally death. The hypoglycemic potential of *Brownea coccinea*, "Rose of the Mountain" was tested over 21 days period. Results were recorded at three days intervals, after establishment of baseline Fasting Blood Glucose (FBG) values for each group. The rabbits were coloured tagged for identification, the control group (group 4) were normal domesticated rabbits, approximately five (5) to six (6) months old. The test subjects that were induced with diabetes, using alloxan, were albino rabbits, approximately five to six months old as well. Alloxan monohydrate used in the present study to induce Type 2 diabetes in 3 months old albino rabbits, resulted in the release of superoxide radicals that destroyed the b-cells in the induced diabetic rabbits. Diabetes, type 1, arises from the destruction of pancreatic b cells, causing a reduction in insulin secretion. The reference drug used in the experiment was glibenclamide, the synthetic hypoglycemic agent and has been used as an antidiabetic drug in Type 2 diabetic patients since 1973 and is still being used.

The rabbits were divided into four groups of three i.e the experiment were done in triplicates. These were group 1, feed only, Group 2, the glibenclamide treated group, Group 3, the group administered with the aqueous extract of "Rose of the Mountains", Group 4, the control group. The results can be discussed: Consider the blood glucose level of rabbits. In general, there was a general decrease in blood glucose level for all four groups. However, these reductions were variable. For Feed only group, there was a reduction from an initial value of (132.33±5.85mg/dl) to the final reading of (80±15mg/dl) on the 21st day i.e 39.55 % reduction. For the Glibenclamide group, there was a reduction from (56.67±3.05mg/dl) to a final value of (45±5.0mg/dl) i.e a 20.59 % reduction. For the induced diabetic group that was treated with the aqueous extract of "Rose of the Mountain", there was a reduction in the blood glucose level from (59±4.0mg/dl) to a final value of (51.67±7.64mg/dl) i.e 12.42% reduction. For the control group, the corresponding values of (58.33± 0.01mg/dl) to (56.33±5.13mg/dl) i.e 3.43% reduction. Based on these values, it can be safely say that the order of decreasing hypoglycemic effect is: Feed only group > Glibenclamide > aqueous extract > control group (Table 1).

Diabetes affect the body weight of an animal and relates to obesity. Obesity generally leads to an increase in body weight. From Table 2, it is evident that there is a general increase in the body weight of the animal and again this is variable, depending on the type of group. For the Feed only group, there was a general increase from (0.47±0.029kg) to a final value of (0.83±0.11kg) i.e a 76.6% increase in body weight. For the glibenclamide treated group, there was an increase in the initial value from (0.52±0.09kg) to (0.73±0.066 kg) i.e a 40.38% increase. For the group that was treated with the aqueous extract, there was an increase from an initial value from (0.48±0.052kg) to a final reading value of (0.79±0.052kg) on the twenty first day i.e 64.58% increase. The control group noted an increase in body weight from an initial value of (0.62±0.115kg) to a final value of (0.87±0.095kg) on the 21st day i.e a 40.32 % increase in body weight. Thus, the increase in body weight followed the trend: Feed only group > Glibenclamide > aqueous extract group Figure 2, graph # 1 shows the average blood glucose of each rabbit/subjects (color represents how they were tagged) over the twenty (21) days period of the experiment verses various treatments. Subjects treated with the drug (Glibenclamide 5mg) had the lowest average of 52.08mg/dL, followed by the control group 54.95mg/dL, then the group given the extract (Rose of the Mountain) 58.29mg/dL, and lastly the group that received no treatment had a reading of 97.08mg/dL. Figure 3, graph # 2 shows the average body weight of the rabbits (subjects) over the twenty one (21) days period. Subjects treated with glibenclamide had the lowest average body weight of 0.593kg, followed by the group given the extract 0.648kg, then the group with no treatment 0.682kg and lastly, the control group 0.724kg. Figure 4., graph # 3 shows the average blood glucose levels of the subjects versus their average body weight. The group that received no treatment had the highest body weight to blood glucose ratio as compared to the other groups. The second highest was the group with the extract "Rose of the Mountain)" followed by the control group and finally, the group that received the drug, glibenclamide.

Anova analyses were done to see whether there was any signicant differences in the Fasting Blood Glucose level (FBG) and Body Weight (BW) between and within groups over the twenty-one (21) days period. The program input values for between groups. However, no values were recorded, within any groups. Table 3. shows that for treatment with aqueous extract of "Rose of the Mountain", there wasn't any significant differences at any one-day measurement as (P > 0.05). For group 2, treatment with Glibenclamide on blood glucose levels, Table 4, there wasn't any significant difference in the blood glucose levels between all groups as (P > 0.05), during the time period, with the exception on Day-12 and Day-15, when the p value were (0.026) and (0.028) respectively, Table 5. shows the Anova analyses for the diabetic rabbit group, that were treated with feed only. There were significant differences between group, for all days of the recordings, as all p-values are < 0.05, with P, ranging from (0.001 to 0.028).

Weight comparison was also done with Anova. Table 6. shows that there wasn't any significant difference in the body weight for the twenty one (21) days period, for treatment with feed alone, as all p-values are greater than 0.05, ranging from (0.482 to 1.00). Similarly, when rabbits were treated with aqueous extract of "Rose of the Mountain", P-values were greater than 0.05 and range from (0.128 to 0.317). For the Glibencamide treated group, Table 7, all P-values are greater than 0.05, indicating no significant difference, an exception to this being P (0.012) on Day-3. An overall Anova one-way analyses were also done Table 8. Table 9 shows that for the group that was subjected to treatment with feed only, P value (0.084) was greater than 0.05, indicating no significant differences. This was further supported by the fact that F (2.7788) is < F critical (3.4668), Table 10 indicating no significant differences. For group 2, treated with Glibenclamide, P- value (0.829) > 0.05 and F (0.188501) < F critical (3.4668) Table 11. For group 3, treated with an aqueous extract of "Rose of the Mountain", there wasn't any significant differences as P-value (0.291317) > 0.05 and F-value (1.308699) < F critical (3.4668). For the control group, the P-value (0.709841) was greater than 0.05 and F value (0.348369) < F critical (3.4668), Table 12.

When Anova, single factor was applied to the body weight, the following were found. For Group 1-feed only, the P-value (0.137947) < P (0.05) and this is further supported by F-value (2.180) < F critical (3.4668), Table 13. For group 2, the glibenclamide treated group, the P-value (0.077115) is < than P = 0.05, and F value (2.902) < F critical (3.4668), indicating no significant differences, Table 14. For group 3, which was treated with aqueous extract, Table 15, there was also no significant differences between group as P-value (0.539502) is greater than 0.05 and F value (0.635602) < F critical (3.4668), indicating no significant differences. For the control group, Table 16.0, the p value (0.01555) < 0.05 and F (5.11) > F critical (3.4668), indicating significant differences.

Conclusion

There seems to be an overall decrease in the blood sugar glucose for all four groups of rabbits, over the twenty one (21) days period. However, this hypoglycemic effect seems to follow the trend: glibenclamide > control group > aqueous extract > feed only group. There also seems to be an increase in body weight of all four four groups of rabbits. Diabetes usually lead to obesity. It was found that the body weight increased according to the trend: glibenclamide > aqueous extract > group feed only > control. The present study showed that aqueous bark extract of "Rose of the Mountain", possessed hypoglycemic and anti-hyperglycemic properties in chemically induced Type 2 diabetic albino rabbits and to a comparable status as that of the synthetic reference compound, glibenclamide. Therefore, its traditional use, as mentioned above is justified and should be added to the list of traditional plants used to combat diabetes.

Acknowledgement

The author thanks his research students for the conduct of this experiment.

References

1. Holleman Frits (2016) Introduction to diabetes mellitus.
2. Poretsky L (2002) Principles of diabetes mellitus. Kluwer Academic, Boston, USA.
3. Diabetes Mellitus (2016) Diabetes.
4. Alberts B, Johnson A, Lewis J, Raff M, Roberts K (2002) Molecular Biology of the Cell. (4th edn.), Garland Science, New York, USA.
5. American Diabetes Association (2003) Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1: S5-S10.
6. Hackett E, Jacques N (2009) Clinical Pharmacist 1: 475-478.
7. Cade W (2008) Diabetes-Related Microvascular and Macrovascular diseases in the Physical Therapy Setting. Physical Therapy 88(11): 1322-1335.
8. American Diabetes Association (2009) Standards of Medical Care in Diabetes. Diabetes Care 32: 1-49.
9. World Health Organization (2015) Diabetes Fact Sheet.
10. Daniels D, Diabetes shattering lives…as too many seek help too late, 2015.
11. Cheng A (2005) Oral antihyperglycemic therapy for type 2 diabetes mellitus. CMAJ 172(2): 213-226.
12. Martins E (2014) The Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. Front Pharmacol 4(00177): 1663-9812.
13. Ngugi MP, Njagi JM, Kibiti CM, Miriti PM (2012) Pharmacological Management of Diabetes Mellitus.
14. Najmi A, Akhtar M, Mohd A, Mujeeb M, Pillai KK, et al. (2012) A pharmacological appraisal of medicinal plants with antidiabetic potential. J Pharm Bioall Sci 4(1): 27.
15. WHO (2016) Genomic resource centre: Genes and human disease.
16. Maritim AC, Sanders RA, Watkins JB (2003) Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 17(1): 24-38.
17. Trevor JA, Katzung, BG, Masters, SB, Katzung & Trevor Pharmacology (1998) Examination and Board Review, Lange Medical Books, McGraw-Hill, USA.
18. Botanic Gardens Conservation International (BGCI) & IUCN SSC Global Tree Specialist Group (2019) Brownea coccinea IUCN Red List of Threatened Species 2019.
19. Sarwar A, Huq TB, Malik T, Kumar Das, Biplab (2015) Antioxidant and analgesic activities of ethanol leaf extract of *Brownea coccniea*. Advanc Medic Plan Res 3(2): 69-72.
20. United States Herbarium (1914–1917) *Brownea coccinea* Jacq". Contributions from the United States National Herbarium. 18: 153 – via Biodiversity Heritage Library; Morton, Julia Frances (1981). Atlas of medicinal plants of Middle America: Bahamas to Yucatan. Springfield, Ill C C Thomas.
21. Shahid W, Durrani R, Iram S, Durrani M, Khan F (2013) Antibacterial activity in Vitro of medicinal plants, Sky Journal of Microbiology Research 1(2): 5-21.
22. Rijo P, Faustino C, Simoes MF (2013) Antimicrobial natural products from Plectranthus plants, Microbial pathogens and strategies for combating them: Science. Technology and Education 922- 931.
23. Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, et al. (2009) Natural Products-antifungal agents derived from plants, Journal of Asian natural Products Research 11(7): 621-638.
24. Jagessar RC, Mohamed N (2010) Antimicrobial activity of selected plants extracts from Guyana's flora. J Pure Appl Micro 4(2): 533-540.
25. Jagessar RC, Allen R (2011) Antimicrobial Potency of the Aqueous Extract of leaves of *Terminalia catappa*. Acad Resea Intern 362-371.
26. Jagessar RC, Mars A, Gomathigayam S (2011) Selective Antimicrobial properties of Leaf extract of *Samanea Saman* against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* using several microbial techniques. Journal of American Science 7(3): 108-119.
27. Jagessar RC, Hafeez A, Chichester M, Crepaul Y (2017) Antimicrobial Activity of the Ethanolic and Aqueous Extract of Passion Fruit (*Passiflora edulis Sims)*, in the absence and presence of Zn (OAc)2.2H2O. WJPPS 6(9): 230-246.
28. Jagessar RC, Hope S (2016) Antimicrobial Activity of the Uncombined and Combined Aqueous Extract of *Phyllanthus Acidus*, *Sphagneticola Trilobata* Leaves and *Doliocarpus Dentatus* Bark against Selective Pathogenic Microorganisms in the absence and presence of Zn2+ cations. WJPPS 5(8): 58-71.
29. Ochwang IDO, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM, et al. (2014) Medicinal plants used in treatment and management of cancer in Kakamega County Kenya. J Ethnopharmacol 151: 1040-1055.
30. Apostolou A, Stagos D, Galitsiou E, Spyrou A, Haroutounian S, et al. (2013) Assessment of Polyphenolic content, antioxidant activity, protection against ROS-induced DNA damage and anticancer activity of Viti vinifera stem extracts. Food Chem Toxicol 61: 60-68.
31. Cao J, Xia X, Chen X, Xiao J, Wang Q (2013) Characterization of flavonoids from *Dryopteris erythrosor*a and evaluation of their antioxidant, anticancer and acetylcholinesterase inhibition activities. Food Chem Toxicol 51: 242-250.

32. Malikovia J, Swaczyynova J, Kolar Z, Strnad M (2008) Anticancer and antiproliferative activity of natural brasinosteroids. Phytochemistry 69: 418-426.

33. Khazir J, Mir BA, PilcherL, Riley DL (2014) Rolke of plants in anticancer drug discovery. Phytochemistry Letters 7: 173-181.

34. Solowey E, Lichtenstein M, Sallo S, Paavilainen H, Solowet E, et al. (2014) Evaluating Medicinal Plants for their anticancer effects. Scientific World Journal 1-12.

35. Krentz AJ, Bailey CJ (2005) Oral antidiabetic agents: Current role in type 2 diabetes mellitus. Drugs 65(3): 385-411.

36. Cheng A (2005) Oral antihyperglycemic therapy for type 2 diabetes mellitus. CMAJ 172(2): 213-226.

37. Ngugi MP, Njagi JM, Kibiti CM, Miriti PM (2012) Pharmacological Management of Diabetes Mellitus.

38. Najmi A, Akhtar M, Mohd A, Mujeeb M, Pillai KK, et al. (2012) A pharmacological appraisal of medicinal plants with antidiabetic potential. J Pharm Bioallied Sci 4(1): 27.

39. Bhutkar MA, Bhise SB (2011) Anti-Oxidative Effect of Tamarindus Indica in Alloxan Induced Diabetic Rats. I J Rese Pharm Biomed Sci 2 (3): 1006-1009.

40. Najmi A, Akhtar M, Mohd A, Mujeeb M, Pillai KK, et al. (2012) A pharmacological appraisal of medicinal plants with antidiabetic potential. J Pharm Bioallied Sci 4(1): 27.

41. Maritim AC, Sanders RA, Watkins JB (2003) Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 17(1): 24-38.

42. Rai, PK, Jaiswal D, Mehta S, Watal G (2009) Anti-hyperglycaemic potential of Psidium guajava raw fruit peel. Indian J Med Res 129: 561-565.

43. Jagessar RC, Rodrigues A, Prashad K, Husain A, Kanhai V, et al. (2018) An investigation of the hypoglycemic effect of the aqueous extract of the fruits of *Psidium Guajava*, *Averrhoa bilimbi* and the peel of *Tamarindus indica* in normoglycemic guinea pigs. J Pharm Drug Deliv Res 7(4): 77-101.

44. Feyzmand S, Shahbazi B, Marami M, Bahrami G, Fattahi A, et al. (2017) Mechanistic *In vitro* Evaluation of *Prosopis farcta* Roots Potential as an Antidiabetic Folk Medicinal Plant. Pharmacogn Mag 13(4): S852–S859.

45. Khan J, Saeed MA, Touqeer S, Adnan S, Masood Z, et al. (2014) Antihyperglycemic, Hypoglycemic and Cytotoxic Activity of *Albizia lebbek* and *Trigonella corniculate*. Research J Pharm and Tech 7(2): 191-195.

46. Hegazy GA, Alnoury AM, Gad HG (2013) The role of Acacia Arabica extract as an antidiabetic, antihyperlipidemic, and antioxidant in streptozotocin-induced diabetic rats. Saudi Med J 34(7): 727-733.

47. Ozbek H, Acikara OB, Ergene B, Ozbilgin S, Kirmizi NI, et al. (2019) Antidiabetic activity evaluation of Onobrychis species on alloxan-induced diabetic mice. Braz J Pharm Sci 55: 2175-9790.

48. Skoog AD, Holler JF, Nieman AT (1998) Principles of Instrumental Analysis. (5th edn.), Thomson Learning, USA, pp. 329-353.

49. Daniel HC (2003) Quantitative Chemical Analysis. (6th edn.), W H Freeman and Company, New York, USA, p. 61-79.

50. Lane DM (2015) Analysis of Variance, USA.