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Establishing the Antidiabetic Potential of Marketed Product Diabex Capsules and Standardization of its Physicochemical Parameters

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ABSTRACT

The present investigation was undertaken with an objective to standardize and validate a self proclaimed proprietary medicine promoted online by the name of Diabex capsule (DC), available for management of diabetes. The procured capsules of DC were evaluated for texture, color, taste and odor. Total ash value of is an indication of the amount of minerals and earthy materials present in the formulations. DC exhibited 9.3 % total ash with 3.16 % acid insoluble ash and 5.08 % water soluble ash. The water soluble and alcohol soluble extractives were 1.48 % and 1.42 % respectively suggesting the formulation to be suitable for human use. The powder of DC was subjected to various chemical tests for preliminary screening of the class of phytoconstituents present in them. The spot for quercetin appeared at R_f value of 0.83 on the TLC plate. The peak at 5.048 min was found due to the presence of quercetin in DC. The quantitation of the quercetin was done from the calibration curve of peak area obtained from standard quercetin and it was found that DC contained 1.89 mg quercetin per 250 mg of DC (0.756 %). A glucose tolerance test determines the blood glucose level in fasting condition then after 2 hours of drinking a solution of glucose in specific quantity. Alloxan is considered to be the most common chemical substance to induce diabetes in experimental animals. DC was able to decrease blood sugar by 35.03% while the standard drug glibenclamide could reduce it by 42.54%. This makes it evident that the polyherbal formulation DC was almost equipotent to the standard drug.

Key words: Antidiabetic, Diabex, Standardization, Physicochemical, Capsule, WHO

1. INTRODUCTION

Diabetes mellitus is a combined pool of diverse disorders usually represents experience of hyperglycemia and intolerance of glucose, due to insufficient insulin production, mall functioning of insulin or both[1]. Such complications produces due to derangements in the regulation systems of storage and mobilization, including the catabolism and anabolism of carbohydrates, lipids and proteins originating from faulty insulin discharge, insulin action, or both[2,3]. Diabetes mellitus is classified on the basis of etiology and clinical presentation, diabetes mellitus is divided in four classes as type-1, type-2, gestational diabetes and other specific types[4]. Type-1 diabetes is said to account for only a alternative of the total burden of diabetes in a population though it is the major type of the diabetes in younger age groups at majority of well-to-do countries[5]. The incidence of type-1 diabetes is increasing in both rich and poor countries[6]. Furthermore, a shift towards type-1 diabetes occurring in children at earlier ages is imminent[7]. A lot of research on finding out of the new generation of anti-diabetic formulation to address the issue is ongoing project[8]. The present allopathic medicines have lots of limitations with other associated difficulties[9]. The diabetes treatment becomes more complicated due to other complications arising with the progression of diabetes[10]. Due to present day work culture, it is very difficult to maintain healthy diet and continuing regular physical activities[11]. Herbal medicine works on multiple mechanisms and there is a probability to cure the disease by curing the root causes of the problem[12].

The primary objective of this work was to standardize an online available polyherbal capsule formulation aspect for quality and viability. Standardization of domestic detailing involves the confirmation of its characterization and assurance of its purity and quality. Standardization of Daibex capsules is not documented. Hence, present study aimed to standardize the Daibex capsules with respect to its organoleptic properties, physicochemical properties and marker quantitation.

2. EXPERIMENTAL DETAILS

Diabex are manufactured and marketed by Dr. Vaidyas-New age ayurveda as herbal product for relief from diabetes. Diabex capsules were purchased from the online store of drvaidyas.com. Quercetin was used as the marker compound and was purchased from Oxford Fine Chemicals Pvt Ltd. All reagents and chemicals used belong to AR grade and purchased from Oxford Fine Chemicals, Mumbai. Experimental animal were procured from approved local breeders.

2.1 Collection of marketed product for standardization

The marketed formulation Diabex capsule was purchased from online store drvaidyas.com. The material was received in packed bottle type container containing 30 capsules. The formulation was abbreviated as DC for study.

2.2 Organoleptic Standardization of DC

Organoleptic properties are those aspects of materials as experienced by senses like sight, taste, smell, and touch in cases where dryness, moisture and stale-fresh factors are to be considered. The organoleptic properties evaluated for DC include Taste, Odor, Color, Texture and size.

2.3 Physicochemical Standardization of DC

Physiochemical studies such as water soluble extractives, alcohol soluble extractives, ether soluble extractives, hydro alcoholic soluble extractives, water soluble ash, total ash, acid insoluble ash, were carried out as per the WHO guide lines. The tablets were powdered using a clean and dry mortar and pestle for determination of the physicochemical parameters.

2.4 Preliminary Phytochemical Screening of DC

Phytochemical screening of DC for determination of Alkaloids, Saponins Glycosides, Flavonoids, Tannins and phenolic compounds, Sterols, Proteins and Amino acids and Triterpenoids, were carried out as per the WHO guide lines.

2.5 TLC analysis of DC and quercetin standard

TLC was developed by Precoated TLC Plate for the standardization of DC. DC powder was extracted with water, followed by petroleum ether and finally with ethyl acetate. The ethyl acetate extract was dried and solubilized in methanol for spotting on the TLC plate. The quercetin standard was also dissolved in methanol and used for spotting on the TLC plate. The developing solvent consisted of tolune-ethylacetate-formic acid (5:4:1) and the developed plate was visualized using iodine vapors[14].

2.6 Quantitative estimation of quercetin in DC

Quercetin in the DC powder was quantified by a HPLC method which involved using a C18 column, UV detector and detection wavelength of 254 nm and flow rate of 0.8 mL/min for the mobile phase comprising of methanol–distilled water–trifluroacetic acid (700 - 300 - 1, v/v/v)[15] The total duration of run was 10 min. Quercetin standard solutions were prepared in methanol and various concentrations of 50, 60, 70, 80, 90 and 100 µg/mL by diluting the stock solution. The powder emptied from DC was dissolved in methanol and filtered. This filtrate was suitably diluted and injected into the HPLC system to obtain the chromatogram.

2.7 Evaluation of antidiabetic activity of DC

2.7.1 Animals

Healthy Wistar rats of either sex, weighing 180-250g were used for the study and housed in polypropylene cages. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [23 ± 2°C] maintained as standard experimental condition. The animals were fed with standard rodent pellet diet and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

2.7.2 Induction of experimental diabetes

For induction of diabetes, animals were subjected to overnight fast (free access to water) for 12 hours to make them additionally susceptible to developing diabetes. Diabetes was induced in the test animals by intraperitoneally administrating alloxan monohydrate (150 mg/kg body weight) solubilized in normal saline. After 72 h mice with blood glucose range of 200 to 350 mg/dl were used for study[15].

2.7.3 Experimental Setup

Animals were categorized into seven groups, each consisting of six rats. Standard pellet diet and water *ad libitum* was provided to the animals.

Group I: Normal healthy rats administered only vehicle (0.5%Tween 80)

Group II: Diabetic control (Alloxan 150 mg/kg)

Group III: Diabetic rats of this group were administered with glibenclamide (10 mg/kg) from 6th day after first administration of alloxan

Group IV: Diabetic rats of this group were administered with DC ethanolic extract (DC 200 mg/kg) from 6th day after first administration of alloxan

2.7.4 Oral glucose tolerance test

Prior to initiation of the experimental procedure, the rats were fed with a bolus of 2g/kg dose of glucose and the level of glucose in blood was estimated at 0, 30, 60 and 120 seconds after administration of glucose using glucometer.

2.7.5 Evaluation of antidiabetic activity

The antidiabetic activity of DC was determined by measuring the blood glucose levels on 1st, 10th and 15th day of administering the extract to the diabetic rats. The decline in glucose level was taken as the indicator for glucose ameliorating potential of the leaf extracts.

3. RESULTS AND DISCUSSION

3.1 Organoleptic Standardization of DC

The content was emptied from the capsule shell and the following observations were made. The results are shown in table below

Table 1: Organoleptic features of DC

Color	Odor	Taste	Texture
Brown powder	Characteristic	Bitter	Coarse

3.2 Physicochemical Standardization of DC

The results of water soluble extractives, alcohol soluble extractives, ether soluble extractives, hydro alcoholic soluble extractives, total ash, water soluble ash, acid insoluble ash are presented in table below:

Table 2: Physicochemical properties of DC

Parameter	Weight of Sample (g)	Weight of ash/extractive (g)	% Value
Total Ash	2	0.186	9.3
Acid insoluble Ash	2	0.063	3.16
Water soluble Ash	2	0.101	5.08
Water soluble Extractives	5	0.074	1.48
Alcohol soluble Extractives	5	0.071	1.42

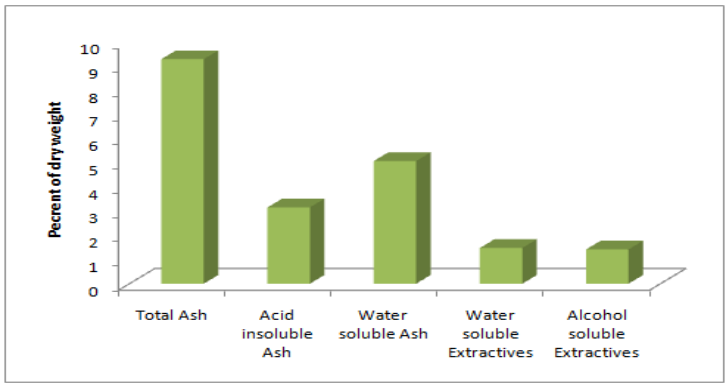

Figure 1: Extractive and Ash values of DC

3.3 Qualitative phytochemical screening

The powder of DC was subjected to various chemical tests for preliminary screening of the class of phytoconstituents present in them. The result is presented in table below:

Table 3: Phytochemical screening of DC

Phytochemical Tested	Observation	Inference
Alkaloid	Cream precipitate formation in Mayer's Test	Present
Glycoside	Greenish color in acetic acid layer in Keller-Killiani Test	Present
Saponin	Frothing Formation	Present
Tannins	Yellow color precipitate in Alkaline Reagent Test	Present
Phenolics	Bluish green color in Ferric chloride Test	Present
Flavonoids	Red color formation in Zinc reduction Test	Present
Proteins and Amino acids	No color formation in Ninhydrin Test	Present
Sterols	Green Color in Burchard Test	Present
Triterpenoids	Grey color in Salkowski Test	Present

3.4 TLC Analysis of DC and Curcumin

TLC analysis of DC was done using quercetin as the marker using tolune-ethylacetate-formic acid (5:4:1) as the developing solvent system. The spots were visusalized using iodine vapors. The spot for quercetin appeared at R_f value of 0.83 on the TLC plate. A spot at the same R_f value was obtained in DC indicating the presence of quercetin in the formulation.

Figure 2: TLC profiling of DC and standard quercetin

3. 5 Quantitation of Curcumin in DC

Quercetin was eluted using HPLC method employing methanol–distilled water–trifluroacetic acid (700 - 300 - 1, v/v/v) as the mobile phase. Standard quercetin was eluted at retention time 5.082 min using the mobile phase. The HPLC chromatogram of DC exhibited peaks at 1.432, 1.998, 5.048, 6.815, 13.865, 14.365, 16.332, 17.532, 18.332 and 21.198 min owing to the presence of several phytoconstituents that could be eluted out using the mobile phase. The peak at 5.048 min was found due to the presence of quercetin in DC. The quantitation of the quercetin was done from the calibration curve of peak area obtained from standard quercetin and it was found that DC contained 1.89 mg quercetin per 250 mg of DC (0.756 %).

This concentration of quercetin might contribute towards various actions elicited by the formulation along with other constituents that are and contribute towards the action of the formulation. The presence of shilajit and amla has been claimed to contribute toward immunity buildup.

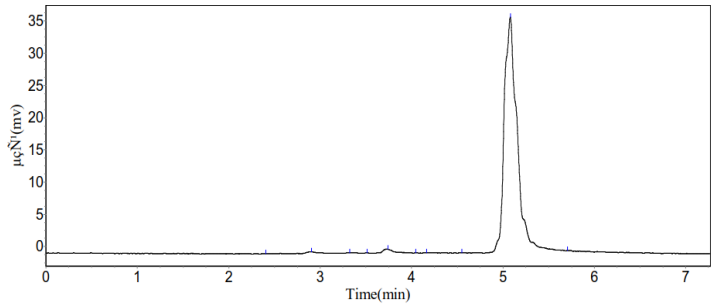

Figure 3: HPLC chromatogram of Quercetin (Retention time 5.082 min, run time 7 min)

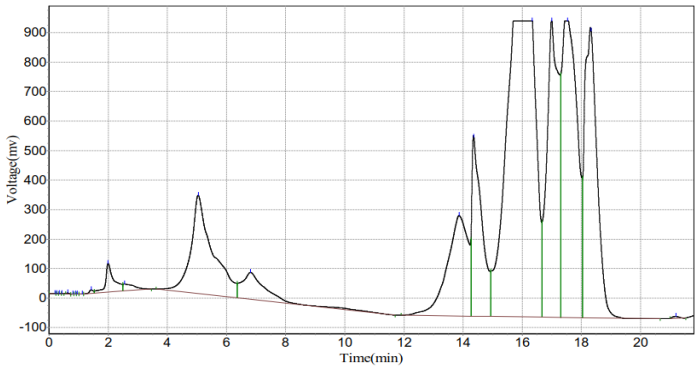

Figure 4: HPLC chromatogram of DC

3.6 Evaluation of antidiabetic action of DC

The effect of DC and standard drug on glucose tolerance as compared to the normal saline control at different hours in alloxan induced experimental diabetes model in rats.

Table 4: Effect of DC on OGTT

Groups	Treatment/ dose	Blood glucose (mg/dl)			
		0 h	0.5 h	1.0 h	2 h
I	Normal control	96 ± 3.1	132 ± 4.8	116 ± 6.9	99 ± 4.5
II	Diabetic control	187.2 ± 2.9	211.9 ± 3.6	229.0 ± 2.01	293.8 ± 2.8
III	Glibenclamide, 10 mg/kg	142.3 ± 1.9	173.8 ± 2.26	169.1 ± 1.5	165.4 ± 2.6
IV	DC 200 mg/kg	164.3 ± 0.9	210.6 ± 1.09	237.9 ± 0.9	194.1 ± 0.78

Values are average ± SD of 6 readings

A glucose tolerance test determines the blood glucose level in fasting condition then after 2 hours of drinking a solution of glucose in specific quantity. The carbohydrates digest into glucose present in daily nutrientstaken.

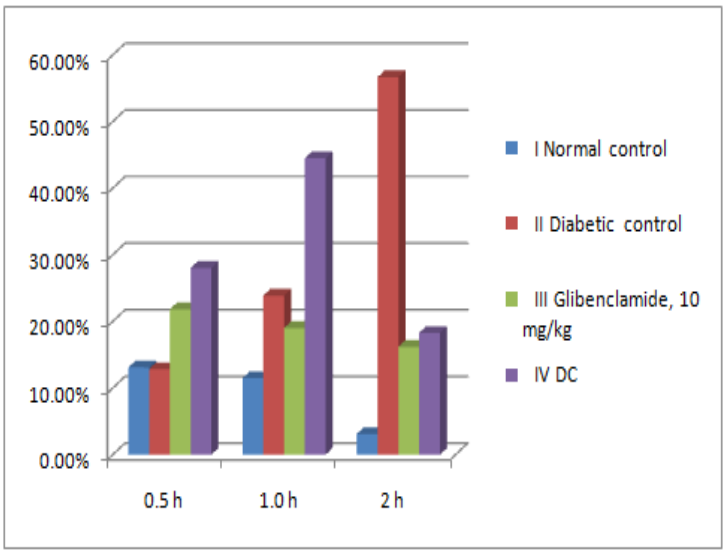

Figure 5: Percent change in glucose levels in DC

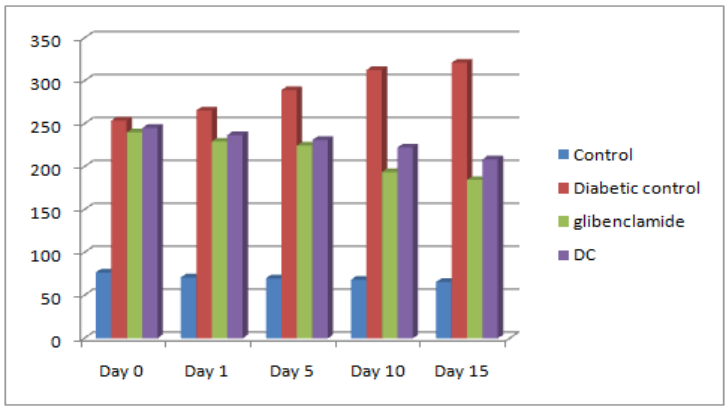

Figure 6: Comparison of blood glucose levels in treatment groups

3.7 The results of antidiabetic activity of DC by alloxan induced diabetic model are shown below

Table 5: Effect of DC on blood glucose

	Level of blood glucose (mg/dl)				
Groups	**Initial**	**Day 1**	**Day 5**	**Day 10**	**Day 15**
Control	76.68 ± 2.06	71.03 ± 3.91	69.86± 3.89	68.23 ± 2.79	65.64 ± 6.58
Diabetic control	253.74± 3.38	265.62± 13.74	289.38± 7.64	312.81± 7.08	321.26 ± 7.14
Gliben clamide	240.02± 2.77	229.36 ± 3.38	224.77± 3.59	193.8 ± 2.80	184.57 ± 2.98
DC	245.29± 4.22	236.94 ± 6.18	231.11± 4.08	222.56± 2.85	208.71± 5.63

Alloxan is considered to be the most common chemical substance to induce diabetes in experimental animals. It has been proven that alloxan can lead to rapid depletion or degeneration of the β cells of the islets of Langerhans thereby causing diabetes[18]. The level of blood glucose was found to decrease significantly in the diabetic rats when compared to control at the end of the 15th day of study. DC was able to decrease blood sugar by 35.03% while the standard drug glibenclamide could reduce it by 42.54%. This makes it evident that the polyherbal formulation DC was almost equipotent to the standard drug.

Insulin-dependent diabetes mellitus was induced by Alloxan administered as injection to animals. Alloxan degrades beta (β) cells partially in the pancreatic islets followed by interruption in quality and quantity of insulin production.

4. CONCLUSION

Plant oriented materials are consumed all over the developing and developed worlds as home based remedies, in OTC drug products and as crude material for pharmaceutical industry and they symbolize a sizeable proportion of the worldwide drug market. Therefore, it is necessary to set up worldwide renowned strategy for assuring quality of plant materials. Herbal drug needs keen observation from collection to finished packaged product to assure efficacy and safety of quality of the product. Adverse actions accounts to regulatory authorities in relation to apply of herbal products are frequently attributable to inferior quality of source material and manufacturing and processing factors, among others. Right recognition of source plant species and selection of suitable plant parts for use as herbal remedies are essential and necessary steps for make sure safety, quality and efficacy of herbal medicines. Therefore, the safety and quality of plant drugs at each stage of manufacturing process have become a chief concern to health care providers, health authorities, herbal industries and public. From the current research different standardization parameters such as physicochemical standards like acid insoluble ash, total ash, alcohol & water soluble extractive values, phytochemical analysis and pharmacological evaluation were carried out. On the basis of results obtained it is concluded that the formulation Diabex capsules contains good characteristics and it may be harmless for human use.

5. CONFLICTS OF INTERESTS

There are no conflicts of interests.

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