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Pharmacognostic analysis and antidiabetic potential of selected marketed polyherbal formulations

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ABSTRACT

Background: Diabetes Mellitus (DM) is a chronic metabolic disorder that is characterized by raised glucose levels. The prevalence of DM is common throughout the world and more especially in Pakistan. Hence, there is an increasing demand for polyherbal formulation (PHF) to treat DM. The increased demand for PHFs has raised questions on their quality. Therefore, quality assurance of these products is of great concern in developing countries like Pakistan. The objective of this study was phytochemical evaluation and comparative evaluation of the antidiabetic activity of PHF. **Methods:** The PHF was collected from the local market. Their quality control parameters, pharmacognostic evaluation, in vivo antidiabetic assay and oral glucose tolerance were evaluated and compared with standard drug metformin. The objective of this study was phytochemical evaluation and comparative evaluation of the antidiabetic activity of PHF. **Results:** In the in vivo antidiabetic assay, the PHFs showed the time and dose-dependent lowering in the blood glucose level of the alloxan (150 mg/kg) rats at days 1, 4, 8, 12, 16 compared to the diabetic control group ($p < 0.001$). zubex, (PHF) showed maximum hypoglycemic effect at both doses (250,500 mg/kg). The ziabeen (PHF) caused lowering in the blood glucose level but not in a significant way. Dawa-e-ajeeb ziabetees showed efficacy at a dose (500 mg/kg) but did not show significant efficacy at a dose (250 mg/kg). PHFs at 250,500 mg/Kg improved oral glucose tolerance overload in rats compared to the hyperglycemic control group ($p< 0.001$), like metformin. **Conclusion:** It was concluded that tested PHFs were of standard quality control, while zubex has maximum antidiabetic activity compared to the standard drug. It is important to check constantly the quality of existing PHFs to ensure their safety and efficacy. In this way, we can reduce the PHFs associated side effects.

Keywords: polyherbal formulation, diabetes Mellitus, pharmacognostic evaluation, hypoglycemia, metformin.

INTRODUCTION

Diabetes Mellitus (DM) is characterized by altered lipids, carbohydrates, and protein metabolism which not only causes hyperglycemia but also results in many complications such as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis [1]. The main cause of the disease is the diminished production of insulin or the tissue becoming resistant to the insulin. Complications such as nerve damage and blood vessel damage can occur. When the disease is poorly controlled, it can cause retinopathy, neuropathy and nephropathy. There is an increase in the number of diabetic patients both in developed and under developing countries [2]. The prevalence of diabetes is four to five times greater in the immigrants south Asians in the United Kingdom. Data from the US Centre for disease control indicates that people with diabetes in 1980 were 5.6 million which reached up to17.1 million in 2007. This increase in diabetes closely relates to obesity [3].

Around the globe, there isan estimated increase in diabetes from285 million in 2010 to 439 million in 2030 [4]. Developing countries show more increase in the prevalence of DM. The increase in obesity in childhood cause increase chances of the occurrence of the disease [5]. There is a high prevalence of diabetes in Pakistan ranging from 7.6 to 11%. The incidence of DM to be estimated was 1.2/100000 per year in the most populated city of Pakistan, Karachi [6]. Gastrointestinal diabetes in Pakistan prevails from 3.2% to 3.5% as compared to developed countries [7].

The disease can be prevented by changing lifestyle and with some drugs such as metformin [8]. It causes reduction in hepatic glucose formation as well as an increase in insulin resistance. But the treatment with metformin causes many side effects related to the gastrointestinal tract (GIT). These side effects sometimes lead to a decrease in the dose of the drug, and even sometimes cause the discontinuation of the course of therapy [9]. However, medicines with herbal origin have been used in the cure of the disorders around the globe because these are considered to have fewer side effects and are free from complications as related to the drugs with synthetic origin [10].

Herbal medicines are used since the early ages of the mankind. The ancient Greek, Chinese, Indians, Egyptians used herbal medicines for the cure of many diseases such as DM. The herbal medicines are integrated part of the culture of the native Africans and Americans. In the history of the sub-continent, the Ayurveda is common system of herbal medicines. In 19th century, due to development of the analytical and modern extraction techniques caused the innovations of the synthetic drugs which cause the decreased usage of the herbal medicines [11].

Recently PHF getting famous due to its advantages which may not have in synthetic ones. PHF has been proven effective to many disorders. Different constituents present in the PHF can cause the desired therapeutic effect and other combinations of herbs potentiate this effect. Many scientists worked on the PHF and prove that these PHF are effective to treat the disorder. Their scientific research had been published in recognized journals [12]. There was a study conducted in the UK, which proved that the usage of the PHF is due to their effectiveness and valuable outcomes [13].

Furthermore, the PHF has a therapeutic range for the cure of the disorders. Even some can cause therapeutic outcomes at low doses and can cause fewer side effects at the high doses. The major problem with the use of the PHFs is that the exact components which exert the effect are not well known. It is most important to know the exact component and the molecular interaction of that compound with other herbs and formulation. But the PHFs are mostly used for the treatment of diseases and they are considered as the safe ones as compared to the synthetic ones [14]. Furthermore, it is needed to standardize the product as well as analysis to ensure efficacy through the clinical trials. Approximately 1200 species have hypoglycemic effects and have been used to cure DM. Furthermore, there are more than 200 natural PHFs are known to have hypoglycemic activity [15].

In the present era, the market of all commodities has become global. Health has become of utmost importance since ancient times. So standardization of these marketed products is necessary to make assure the uniform product. Standardization assures a consistently stronger product with guaranteed constituents. Chemical and instrumental analysis is routinely used for analyzing the single herbal ingredient drug for standardization. The purpose of this study was to collect the PHFs from the market, and evaluate their quality, determined their antidiabetic activity and to compare them with the standard drug metformin.

MATERIALS AND METHODS

Sampling

Packs of 120 tablets of polyherbal formulations D1, D2 and D3 were purchased from the local market. The detail of collected PHFs has been shown in table 2.1

Table 2.1. Marketed polyherbal formulations with manufacturer.

Sr. No.	Drug name with code	Manufacturer's name	Batch No.	Expiry date
1.	Zubex (D1)	Quershi Industries (Pvt.) Ltd.	190815	07-21
2.	Ziabeen (D2)	Ashraf Laboratories (Pvt.) Ltd.	19215	03-21
3.	Dawa-e-ajeeb ziabetees (D3)	Ajmal Dawakhana (Pvt.) Ltd.	A1123	07-21

Determination of quality control parameters

The quality control parameters such as weight variation test, friability test, disintegration test were performed according to the pharmacopeia (USP 36 NF31, 2013).

Phytochemical screening

Different constituents like alkaloids, phenols, steroids, proteins and tannin were identified through standard protocols [16].

Pharmacognostic evaluation

Total ash, acid insoluble ash and water-soluble ash were determined through standard protocols [17].

In vivo antidiabetic assay and body weight measurement

Animals

Adult Albino rats of both genders were retained in well-ordered temperature control (20–25◦C) at the Riphah Institute of Pharmaceutical Sciences, Islamabad, Pakistan animal house. Animals had free accessed to standard diet and water ad libitum. Experiments were performed with verdicts of the Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council (1996), approved by the Research Ethics Committee (REC) of Riphah Institute of Pharmaceutical Sciences Islamabad (Rec/Rips/2018/2020).

Blood glucose level and body weight monitoring

The Wistar albino rats of either sex aged 4 months (body weight 180 g) were divided into nine groups, each group contained five rats. Group 1 received normal saline. Group 2 received standard hypoglycemic agent metformin of lethal dose 500 mg/kg. Group 3 acts as non-diabetic control. Group 4, Group 5, Group 6, Group 7, Group 8 and Group 9 were received D1, D2 and D3 at 500 mg/kg and 250 mg/kg respectively. Wistar rats fasted overnight for 12-16 h. Hyperglycemia was introduced in these rats by the intra-peritoneal injection of ice-cold aqueous alloxan monohydrate (150 mg/kg) [18].

Oral glucose tolerance test (OGTT)

Albino rats (weight 180 g) were taken and divided into nine groups, each group contained five rats. Group 1 was nondiabetic control and received the normal saline 10 mg/kg. Group 2 was the diabetic control and received the alloxan 150 mg/kg. Group 3 was positive control and received the metformin 200 mg/kg. Group 4, 5, 6, 7, 8, 9, were D1, D2 and D3 treated at two doses 250 mg/kg and 500 mg /kg. The duration of the pre and post treatment was 30 mins. The dose of glucose was 3 g/kg which was used as a loading dose. For the determination of blood glucose level Accu check instant glucometer was used and the interval was of 0, 30, 60, 90 and 120 mins.

RESULTS

Quality control parameters

Three different types of PHFs were taken and all in vitro tests were performed which complied with the USP 2013. These tests include the weight variation test, hardness test, disintegration test and friability test.

Weight variation test

From each PHF twenty tablets were taken and weighed and their average weight was calculated. It was noted that all PHFs were in the specified limit which such as ±5%. The results of the weight variation test has been shown in Table 3.1.

Table 3.1. Result of weight variation test

Sr. No	PHF	Average weight	Average Deviation	Limit
1	D1	547.00	0.0028	5%
2	D2	506.75	0.0022	5%
3	D3	526.45	0.0113	5%

Hardness test

For the determination of hardness, Monsanto hardness tester was applied and the strength of the individual tablet was tested for each PHFs. The results for the hardness test has been shown Table 3.2.

Table 3.2 Result of hardness test

S. No	PHF	Average Hardness (kg/cm2)	Limit (Kg/cm2)
1	D1	8.1	5-10
2	D2	11.8	5-10
3	D3	9.2	5-10

Disintegration test

For the disintegration test, six tablets from each PHFs were taken and the test was performed. The temperature for this test was maintained at 37 °C. The tablet should compel with the test limit time which was 30 minutes. The result for the disintegration test has been shown in Table 3.3.

Table 3.3 Disintegration time of the PHFs

Sr. NO.	PHF	Average disintegration time (Minutes)	Limit (Minutes)
1	D1	7.6	30
2	D2	3.4	30
3	D3	5.2	30

Friability test

For the friability test, the Roche friabilator was used. For the test 20 tablets from each PHF were selected and weighed. Tablets were going under shock as they were fall from the 6 inches' height. After the 100 revolutions the weight was measured again.. The acceptable limit for this test is less than 0.8 percent. The result for the friability test has been shown inTable 3.4

Table 3.4 Result for friability

Sr. No.	PHF	% weight loss	Limit
1	D1	0.40	0.8
2	D2	0.31	0.8
3	D3	o.43	0.8

Phytochemical screening

Phytochemical analysis of the PHFs revealed the presence of Alkaloids, tannins, saponins, flavonoids, glycosides and sugars.

Pharmacognostical evaluation

Pharmacognostic evaluations like total ash value, acid insoluble ash, water-soluble ash and sulphated ash were determined and their results has been shown in Table 3.5.

Table 3.5. Pharmacognostical evaluation of PHFs

Treatment	D1	D2	D3
Total ash value	13%w/w	14.5%w/w	16.7%w/w
Acid insoluble ash	0.5%w/w	0.5%w/w	0.5%w/w
Water soluble ash	4.5%w/w	5%w/w	4.7%w/w
Sulphated ash	2.4%w/w	2.5%w/w	2.3%w/w

Effect on Blood Glucose Level

For each group the blood glucose level was determined at the days 1, 4, 8, 12 and 16 and their results has been shown in Figure 3.1 and Table 3.6.

Table 3.6. Blood glucose levels at different treatment days.

Treatment	Day 1	Day 4	Day 8	Day 12	Day 16
Non Diabetic Control (Saline 10 ml/kg)	77.60±2.24	77.8±2.77	76.8±1.46	78.4±2.65	76.8±1.31
Diabetic Control (Alloxan 150 mg/Kg)	360.0±13.9	376.2±3.2	410.5±9.6	436.2±8.5	484.2±37
Alloxan (150mg/kg) + D1(250 mg/kg)	419.6±17.7,	197.8±40.1	141.4±13.5	118.0±8.6	96.8±1.9

Alloxan (150 mg/kg) + D2(250 mg/kg)	427.4±40.5	300.4±67.0	, 154.0±39.9	148.2±28.9	117.0±10.4
Alloxan (150 mg/kg) + D3(250 mg/kg)	406.0±46.7	212.0±44.6	64.2±38.41,	127.0±19.2	121.2±20.5
Alloxan (150 mg/kg) + D1(500 mg/kg)	433.0±6.37	169.4±28.1	149.4±8.0	101.4±5.3	89.8±2.7
Alloxan (150 mg/kg) + D2(500 mg/kg)	411.2±33.7	342.4±4.3	269.0±32.7	152.0±19.7	145.8±19.6
Alloxan (150 mg/kg) + D3(500 mg/kg)	408.0±18.27	219.8±34.25	106.4±4.0	95.6±2.52	91.8±3.63
Alloxan (150 mg/kg) + Metformin (200 mg/kg)	364.75±9.6	156.0±33.7	87.5±2.39	88.7±2.68	90.7±0.4

Data presented as mean ± SEM. N= (5).

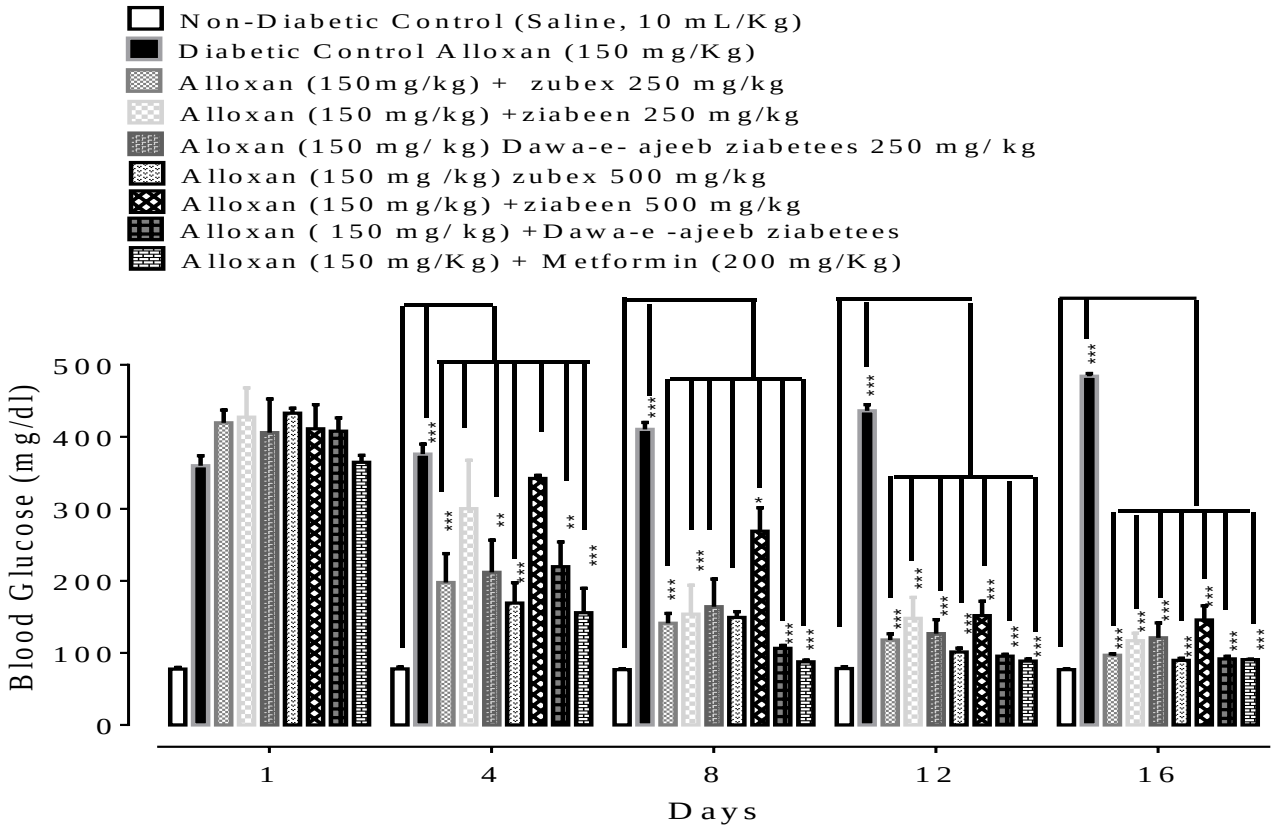

Figure 3.1. Bar-graph representing blood glucose levels at different treatment days of saline treated group (non-diabetic control), alloxan treated group (diabetic control), inhibitory effect of zubex, ziabeen, Dawa-e-ajeeb ziabetees at different doses (250, 500 mg/Kg) and metformin treated group against alloxan-induced hyperglycemia in rats. Data presented as mean ± SEM. Statistical analysis used one-way ANOVA, followed by post-hoc Tukey's test *p < 0.05, **p < 0.01 and ***p < 0.001 comparison of the blood glucose levels of carveol and metformin treated groups vs. diabetic control group. The sample size in each group comprised of 5 rats.

Effect on weight variation

The effect of the respective PHF on the weight variations was studied on the days 1, 4, 8, 12, 16 and variations were noted. The results for Diabetic Control (Alloxan 150 mg/Kg) at different days were 201.8±1.57, 177.1±3.41, 163.4±1.83, 152.6±1.91 and 152.6±1.91. The results for Alloxan (150mg/kg) + D1 (250 mg/kg) at day1, day4, day8, day12 and day16 were 203.8±8.5, 174.9±2.06, 170.1±2.04, 166.7±2.0 and 167.6±1.82. The results for Alloxan (150 mg/kg) +D 2(250 mg/kg) at day1, day4, day8, day12 and day16 were 191.6±9.17, 176.1±5.81, 174.4±5.28, 173.0±5.49 and 172.3±5.87. The results for Alloxan (150 mg/kg) + D3 (250 mg/kg) at day1, day4, day8, day12 and day16 were 205.4±2.87, 193.2±6.53, 192.4±7.42, 190.2±7.49 and 194.6±8.52. The results for Alloxan (150 mg/kg) + D 1(500 mg/kg) at day1, day4, day8, day12 and day16 were 205.4±2.87, 193.4±6.44, 194.2±6.88, 193.6±6.03 and 196.0±6.45. The results for Alloxan (150 mg/kg) +D 2(500 mg/kg) at day1, day4, day8, day12 and day16 were 214.2±0.37, 198.0±3.74, 189.8±5.93, 190.5±3.82 and 186.4±2.20. The results for Alloxan (150 mg/kg) + Metformin (200 mg/kg) at day1, day4, day8, day12 and day16 were 173.6±6.61, 157.4±5.23, 158.6±5.40, 159.4±4.98 and 158.0±5.06 (Table 3.6)

Table 3.7. Effect of PHFs and metformin at different treatment days on body weight (g) of alloxan-induced diabetic rats.

Treatment	Day 1	Day 4	Day 8	Day 12	Day 16
Diabetic Control (Alloxan 150 mg/Kg)	201.8±1.57	177.1±3.41	163.4±1.83	152.6±1.91	152.6±1.91
Alloxan (150mg/kg) + zubex(250 mg/kg)	203.8±8.5	174.9±2.06	170.1±2.04	166.7±2.0	167.6±1.82
Alloxan (150 mg/kg) + ziabeen(250 mg/kg)	191.6±9.17	176.1±5.81	174.4±5.28	173.0±5.49	172.3±5.87
Alloxan (150 mg/kg) + Daw-e-Ajeeb ziabetees (250 mg/kg)	205.4±2.87	193.2±6.53	192.4±7.42	190.2±7.49	194.6±8.52
Alloxan (150 mg/kg) + zubex(500 mg/kg)	205.4±2.87	193.4±6.44	194.2±6.88	193.6±6.03	196.0±6.45
Alloxan (150 mg/kg) + ziabeen(500 mg/kg)	214.2±0.37	198.0±3.74	196.8±3.91	194.4±3.76	192.0±4.52
Alloxan (150 mg/kg) + Dawa-e-Ajeeb ziabetees (500 mg/kg)	211.8±3.0	188.2±5.31	189.8±5.93	190.5±3.82	186.4±2.20
Alloxan (150 mg/kg) + Metformin (200 mg/kg)	173.6±6.61	157.4±5.23	158.6±5.40	159.4±4.98	158.0±5.06

Data presented as mean ± SEM. N= (5).

Effect on the oral glucose tolerance

Blood glucose level of the different groups at the different time intervals was determined and the results are represented below Figure 3.2 and Table 3.8.

Table 3.8. Oral glucose tolerance at different time interval.

Treatment	0 minute	30 minute	60 minute	90 minute	120 minute
Non Diabetic Control (Saline 10 ml/kg)	98.2±2.20	184.2±7.88	154.4±6.76	140.2±9.48	116.6±7.35
Diabetic Control (Alloxan 150 mg/Kg)	156.2±4.46	204.2±3.95	206.6±9.50	205.8±0.58	205.6±2.82
Alloxan (150mg/kg) + D1(250 mg/kg)	174.0±20.60	136.80±9.76	125.60±8.18	112.20±6.63	101.8±4.46
Alloxan (150 mg/kg) + D2(250 mg/kg)	148.2±17.25	149.2±8.04	135.2±6.68	106.0±4.90	98.6±3.88.
Alloxan (150 mg/kg) + D3(250 mg/kg)	126.8±8.65	137.0±4.06	122.2±4.14	111.0±1.78	99.44±1.77
Alloxan (150 mg/kg) + D1(500 mg/kg)	134.2±11.33	127.2±12.45	137.8±2.02	118.60±3.07	108.80±5.05
Alloxan (150 mg/kg) + D2(500 mg/kg)	128.8±7.95	138.4±3.32	123.0±4.21	113.8±1.82	79.6±18.9
Alloxan (150 mg/kg) + D3(500 mg/kg)	133.4±11.72	128.4±01.96	141.2±9.06	120.40±4.25	108.2±5.60
Alloxan (150 mg/kg) + Metformin (200 mg/kg)	115.2± 5.61	171.4±5.68,	138.8±3.38	111.8±4.85	87.6±3.14

Data presented as mean ± SEM. N= (5).

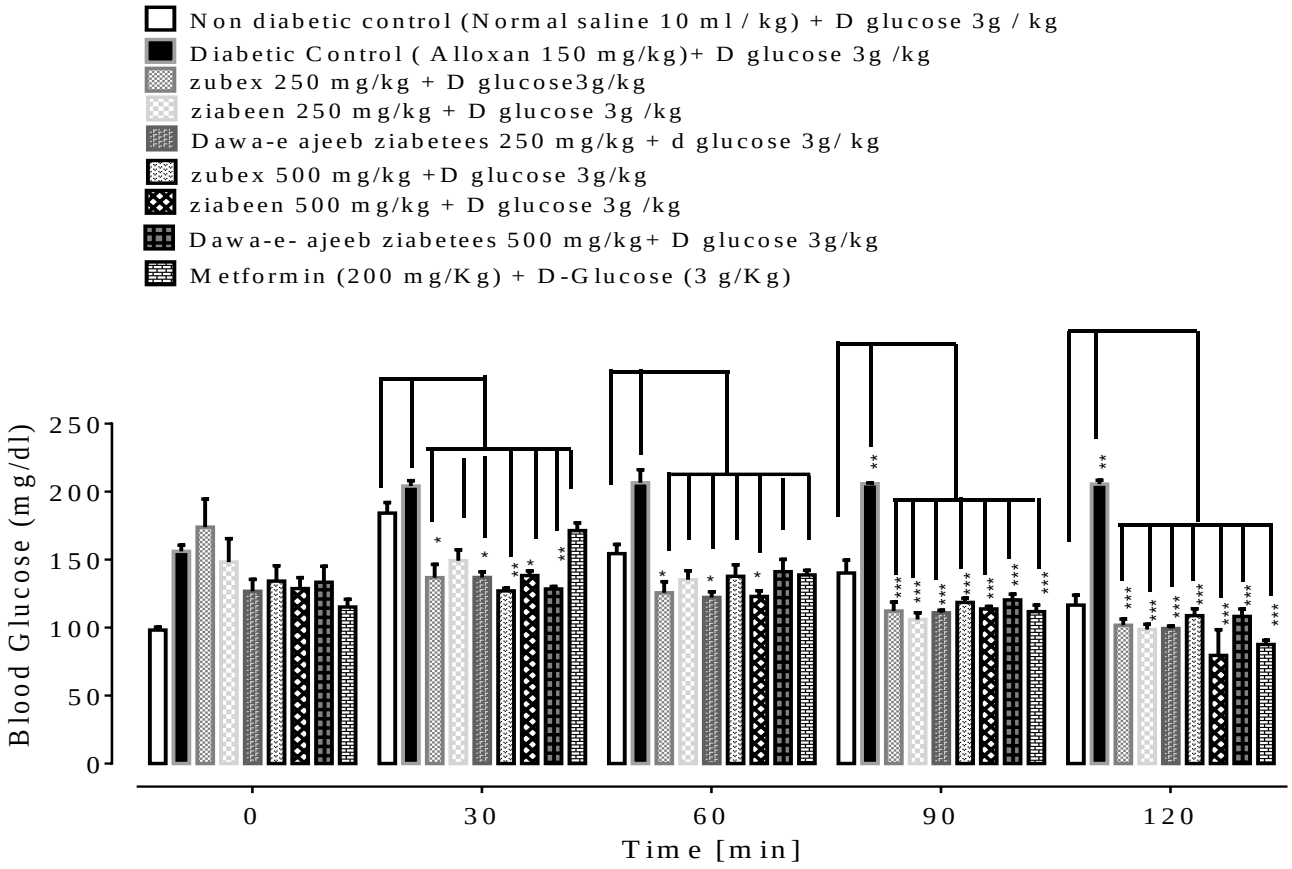

Figure 3.2. Bar-graph representing blood glucose levels at different treatment minutes of saline treated group (non-diabetic control), alloxan treated group (diabetic control), inhibitory effect of zubex, ziabeen, and Dawa-e-ajeeb ziabetees at different doses (250, 500 mg/Kg) and metformin treated group against alloxan-induced hyperglycemia in rats. Data presented as mean ± SEM. Statistical analysis used one-way ANOVA, followed by post-hoc Tukey's test *p < 0.05, **p < 0.01 and ***p < 0.001 comparison of the blood glucose levels of PHFs and metformin treated groups vs. diabetic control group. The sample size in each group comprised of 5 rats.

DISCUSSION

To the best of our knowledge, for the first time we perfomed Pharmacognostic analysis and antidiabetic potential of selected marketed polyherbal formulations. Diabetes is associated with obesity, so PHFs can be used as the drug of choice for DM. Metformin also possesses antidiabetic as well as anti-hyperlipidemic [19]. In the present study it was noted that PHFs act as the antidiabetic agent and their effects are somehow compared to the effects of the metformin. There was a significant lowering in the blood glucose level for the D1 at dose 250 mg/Kg and 500 mg/Kg, which was 96.8±1.9 and 89.8±2.7 respectively, and the blood glucose level for the metformin was 90.7±0.4. By comparing these results, it is concluded that D1 is as significant for the treatment of DM as metformin.

Another drug D 2 showed a decrease in the blood glucose level and the results for doses 250 mg/Kg and 500 mg/Kg were 117.0±10.4 and 145.8±19.6 respectively. By comparing metformin and ZBX, it showed less efficacy in lowering the blood glucose level. For the drug D 3, at dose 250 mg/Kg and 500 mg/Kg, the results were 121.2±20.5 and 91.8±3.63 respectively. By comparing with metformin it is noted that it has significance at the dose of 500 mg/Kg.

The decrease in the weight is noted on different days reveals that the most likely decrease in weight occurs during the treatment with PHF D 3. By comparing with the Metformin results it is concluded that decrease in the weight that occurred during the treatment with the PHFs, is not more than the Metformin.

Pharmacognostic evaluations such as the ash values are used to find the purity and quality of the drug. It indicates the existence of the different impurities such as carbonates, oxalates and silicates. The water-soluble ash is used to find the amount of the inorganic compounds. The acid-soluble ash mainly determines the silicates. The results of the pharmacognostic evaluation reveal that drugs are mostly free from impurities.

The quality control parameters such as hardness, friability, disintegration also reveal that PHFs are of uniform quality. The results obtained from the quality control tests revealed that the tested drugs are of suitable quality. In order to further validate the PHFs potential benefits, more research is required. Because many studies already conducted are of poor quality, this must be addressed by implementing corrective measures for research trials. Quality and standardization of herbal medicine control is possible but difficult to achieve. Herbal diabetes medications come in a variety of forms. When compared to synthetic medications, it's a win-win situation. Aside from that, herbal compositions are governed differently across the country [20].

The "Prakriti" is well known in Ayurveda for determining the efficacy of various herbal treatments, as well as a key factor to be included in clinical trials as an inclusion/exclusion criterion. The application of the same in clinical trials would result in a better outcome of Ayurvedic treatments, which would aid in the development of a successful trial protocol. It is critical to calculate the formulation dose based on whether the crude drug or extract is utilised, as well as the extract dose based on the extractive value [20, 21].

In general, Ayurvedic practitioners prescribe the dose of medication which is based on certain features of the patient's body, disease diagnosis, and treatment scenario. It is important to mention that the patients should be prescribed and given the exact dose of medicine according to study protocol in clinical trials which should be thoroughly checked by patient compliance on regular basis [22].

The placebo-controlled, double-blind randomised trial may be considered the gold standard in herbal medicine safety and efficacy investigations. Furthermore, the findings of herbal medicine in randomised clinical trials may go a long way toward revealing new knowledge that will improve everyone's health [23].

CONCLUSION

The present study reveals that D 1, a PHF has maximum anti-diabetic effect at doses (250,500 mg/kg). The other PHFs such as the D 2 had shown the effect less than as compared to the metformin at both doses (250,500 mg/kg) and D 3 also showed the antidiabetic effect as compared to the metformin at dose (500 mg/kg), and at dose (250 mg/kg) it showed a less significanteffect..It wasconcluded that PHFs can be used for the treatment of the DM, but it is a need of time to continue working on adose-dependent effects and quality control parameters of the PHFs to ensure that these PHFs can also be used as the alternative treatment.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHOR CONTRIBUTION

All author contributes equally.

FINDING SOURCE

This study is not funded by any organization

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