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In-vivo Hypoglycemic Activity of *Grewia asiatica* Fruit Extract in Streptozotocin Mediated Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ZA, AN and MH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SA and UGF managed the analyses of the study. Authors AA and WAS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Diabetes mellitus has high global prevalence and occurrence and is considered to bean endocrinological and/or metabolic disorder. Conventional drug treatment is costly and has toxic side effects, although it is successful in treating diabetes mellitus. If effective and less toxic, herbal medicine will thus include alternative therapy. This research has been designed to investigate the role of Grewia asiatica extract in the control of diabetes in male albino rats with Streptozotocin mediated type 2 diabetes. Grewia asiatica fruit extract at a dose of 200mg/kg was given to

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Streptozotocin mediated type II DM Rats. A known anti-diabetic drug, Glibenclamide has been used as a standard drug. The method of the research was to monitor the effect of Grewia asiatica on the blood glucose level of Rats. In this study, Rats were split into four categories i.e. Control, Streptozotocin treated, Streptozotocin + Glibenclamide treated and Streptozotocin +Grewia asiatica extract-treated group. Grewia asiatica fruit extract significantly improve the blood glucose levels as compared to the standard drug Glibenclamide in Streptozotocin mediated diabetic group.
Conclusion: It was concluded that Grewia asiatica may be used in the treatment of diabetes or decreasing the elevated level of blood sugar.

Keywords: Streptozotocin; diabetes mellitus; glibenclamide; Grewia asiatica.

1. INTRODUCTION

Diabetes is classified as a recurring or a chronic, dysmetabolic syndrome usually permeated by elevated levels of blood sugar i.e. glucose, either due to the improper uptake of insulin by pancreatic cells or consequently due to complete deprivation of insulin [1]. Many substantial complications are also correlated with this metabolic syndrome and one of the accentuate is the premature death, but people with such disorders can adopt some measures either to control or demote the associated complications patently [2]. Though Diabetes Mellitus has devised ages back, yet its prevalence has now extended to an astounding level, with an overall incidence of affecting 151 million individuals each year globally [3]. The prevalence is highest in Asia where approximately 44 million patients are reported as diabetic each year. The others affected continents include Europe with a ratio of 8.5 million reporting and 2.5 million in the African mainland [4].

As per the predictive warning of President of International Diabetes Foundation (IDF), Ms de Alva, in 21st-centuryDiabeteswill be one of the strenuous health challenges. This is the most alarming condition especially in developing nations, due to inadequate evaluation or detection, or unavailability of Insulin or other healthcare accesses that may worsen the situation. The cynosure of IDF as per the President-Elect, Professor Sir George Alberti is particularly on developing countries as they are one of the finest advocates for diabetes incidence [5].

At the end of 20th century, a prediction was given by WHO regarding the drastic elevation in case reporting of diabetes i.e. 125 million in 1995 to an estimate of 300 million by 2025.[3] As per the statement of Dr Crook, considering last few centuries, Diabetes has been marked as one of the rare health disorder in the African region, as it is uncommon but the overall consequences are fatal.[6] Nevertheless, as per the epidemiological research and experimentation did in the 20th century, the plotted frame is completely different than expected, as the characterization of DM and prevalence has elevated the burden of non-communicable disease states.[7,8] To date, the International Diabetes Federation (IDF) have estimated that 451 million adults live with diabetes worldwide in 2017 with a projected increase to 693 million by 2,045 if no effective prevention methods are adopted.[9]

Heredity is one of the main consideration in the advancement of DM. If both the parents have type II diabetes, its transfer and prevalence will ultimately result in their children. In the case of type I diabetes, less than 20% possibilities are there for genetic transmission. If one of the monozygotic twins suffers from diabetes type II, odds are that almost 100% the other twin will likewise develop it. Whereas in case of diabetes type I, if one of the identical twins develops such a disease, there are only 40-50% chances that the other twin will develop so. This indicates that Genetics and Inheritance is one of the major factors for this disorder along with other leading factors such as food or type of diet, surrounding environment, other diseases, viral infections etc. [10]

The associated complications for diabetes include neuropathy, retinopathy, nephropathy, angiopathy, ketoacidosis, and elevated susceptibility to infections, hyperglycemic hyperosmolar non-ketonic coma, and hyperlipidemia. The consequences of these complications may result in reduced lifespan, associated disabilities, as of eyes, and a large cost for its treatment. [11,12]

Considering the therapy options for DM, one of the major choice is the change in lifestyle i.e. physical activities, weight management, nutritional plan, and oral anti-diabetic drugs or insulin remedial use.[13] The overall result of this lifestyle management is correlated with many

side effects, isn't cost-effective and requires health-staff expertise.[2] To avoid these adverse consequences, and produce cost-effective therapies, a switch towards natural substances is being preferred rather than the use of synthetic medicinal therapies.[14] For this purpose, many species of the plants are being used, to treat the life-threatening diseases, yet with minimal side effects and costs. One such disease being treated is Diabetes since antiquity, and some recent researches have confirmed the efficacy of various plant preparations for diabetes with increased efficacy and decreased toxicity.[2]

Grewia asiatica from family Tiliaceae, genus Grewiais a shrub majorly distributed in the warmer region.[15] In Pakistan, 10 varieties of genus Grewia, have been recognized as *Grewia asiatica* L., *Grewia glabra* Blume, *Grewia damine* Gaertn, *Grewia tenax* (Forssk.) Fiori., *Grewia elastic* Royle., *Grewia sapida* Roxb., *Grewia helicterifolia* Wall., *Grewia optiva* J. R. Drumm. ExBurret*, Grewia microcos* L. and Grewia*villosa*. The plant is cultivated in southern Asia, i.e. in Pakistan, East Combodia, and is native to other Tropical regions. According to a research of Journal of Medicinal Plants, botanical features of *Grewia asiatica*, are significant in regards to nutritional value.[16,17] It is reported to have a minimal amount of calories and fat but is regarded as rich in vitamins, fibers and other minerals.[18] Many types of research have proved various therapeutic and pharmacological effects from different parts of *G. asiatica*, such as properties against microbes, cancer, and anti-platelet effects have been observed upon studies on its leaves whereas its fruit possesses Ethno-botanical properties.[19] The main use of fruit is associated with its properties such as Stomachic, Anti-tumor, Anti-cancer, Astringent whereas the unripe form is usually used for its Anti-inflammatory, and Anti-pyretic activities.[20] It is also used in respiratory tract disorders, and cardiac as well as blood disorders.[21]

This research aimed to evaluate and propose the medicinal value, potential therapeutic uses, and pharmacological activities of *Grewia asiatica* by an in-vivo procedure for Diabetes Mellitus.

2. MATERIALS AND METHODS

2.1 Plant Material

Grewia asiatica fruit (5 Kg) were obtained from the genus Grewia orchid, Dera Ismail Khan, Pakistan. Pharmacognosy department under the Faculty of Pharmacy, University of Karachi, identified the plant.

2.2 *G. asiatica* Fruit Extract Preparation

Seeds of *G. asiatica* are removed and fruits are washed. Ethanol (95%) is mixed in the crushed juicy pulp of each fruit. This technique is rehashed threefold. By using a rotary evaporator, under reduced pressure, the crude extract is filtered and evaporated [22]. For additional utilization, it is kept in the refrigerator.

2.3 Experimental Design

A total of 144 rats were alienated into four sets in this study. Group A, considered as control; Group B, as a positive control; Group C, as GLB group; and Group D, as a tested group. All four sets comprising 12 male albino rats. The test was rehashed three times.

Group A: which is considered as a control group received distilled water per oral for 21 days.

Group B: Rats of this set received Streptozotocin (30 mg/Kg) as a single dose through intraperitoneal injection [23].

Group C: Rats of this set (Streptozotocin mediated diabetic rat) received Glibenclamide 0.5 mg/ kg for 21 days per oral administration[24].

Group D: Rats of this set (Streptozotocin mediated diabetic rats) received ethanolic *Grewiaasiatica* fruit extract at a dose of 200mg/Kg peroral administration [25].

2.4 Estimation of Blood Glucose

In vacutainer containing gel tubes through cardiac puncture technique, the blood samples were collected [26]. For 10 minutes rotations at 4000 rpm the serum was separated by centrifugation. Blood Glucose was checked by using the Cobas ® Gluco-quant Glucose /HK kit for Glucose (GLU).

2.5 Determination of Blood Glucose Level

After one week of streptozotocin induction, research animals were fasted for (12-16 hrs) to determine blood sugar level by taking of blood from the tail vein of rats. Blood sugar level (plasma) in between 100-150 gm/dl or above.

We performed an oral glucose tolerance test for confirmation of the induction of hyperglycemia.

2.6 Oral Glucose Tolerance Test

Animals were fasted for (12-16 hrs) to perform an oral glucose tolerance test. To confirm the induction of hyperglycemia, we performed an oral glucose tolerance test by giving 1 gm/kg of glucose through the oral route. Before loading the glucose syrup baseline blood glucose value at 0 hr was determined. This was followed by blood glucose determination via glucometer (ACCU-CHEK® Active, Ser.No. GU07796823, Roche, Mannheim Germany) at time intervals of 30, 60, 120, and 180 mins. Following the progression of diabetes, the rats having moderate diabetes with hyperglycemia and glycosuria considered as diabetic and utilized for treatment with *Grewia asiatica* extract and standard drug glibenclamide for 21 days by the oral route. After 21 days of treatment checked blood sugar level to observe the anti-diabetic effect of drugs by taking the blood from cardiac puncture of rats.

2.7 Statistical Analysis

All the quantitative outcomes were investigated statistically by SPSS software version 21. Results were contrasted with control values by applying ANOVA, considered p <0.05 value as significant.

3. RESULTS

3.1 Blood Glucose Levelestimation

3.1.1 Blood glucose level determination via oral glucose tolerance test

OGTT test outcomes to confirm the induction of diabetes by administering 1 gm/kg of glucose orally to male albino rats were shown in Table 1, Fig. 1. After 30min of glucose administration, there was a remarkable elevation in blood glucose level.

3.1.2 Glibenclamide and *Grewia asiatica* extract effects on blood glucose in rats

Albino rats of weight 200± 20 g were chosen for the study to examine the outcomes of Glibenclamide and *Grewia asiatica* on blood glucose. Rats' blood sample was taken to evaluate glucose levels. The animals have treated with *Grewia asiatica* at a dose of 200mg/kg and Glibenclamide with 0.5 mg/kg for21 days through oral route of administration. The assessment of blood glucose was done by ANOVA SPSS version 21.

3.1.3 Glibenclamide effecton rats blood glucose level

In this research study, the control group blood glucose level was 126.6 ± 3.21 showed in table 2. While the streptozotocin mediated diabetic group blood glucose level was 432.08±10.654and the rats treated with Glibenclamide blood glucose level was 108.50±2.14 *** showed highly significant results.

3.1.4 *Grewia asiatica* effect on rats blood glucose

In this study, the control group blood glucose level was 175 ± 3.07whereasdiabetes-induced group blood glucose level was 395.145 ± 9.372. The treated group of *Grewia asiatica* was showed the blood glucose level 115.0 ± 2.67*** presented in table 3 which was found highly significant.

3.1.5 Correlation of blood glucose level between control, diabetic, standard, and treated group

The *Grewia asiatica* treated group blood glucose level was 115.0 ± 2.67** presented in Table 1 Fig.2, whereas the Glibenclamide standard group

Table 1. Blood glucose level determination via oral glucose tolerance test

Time	Blood sugar level [Mean ± SEM]
0- Minutes	246.42±14.905
30- Minutes	424.08 ±13.838
60- Minutes	368.49±10.385
120- Minutes	315.05±11.532
180- Minutes	265.42 ±4.801

Values are shown as mean along with standard error of the mean (mean ± SEM) n= each set comprising 12 rats. Level of significance p < 0.05, p < 0.01**, p < 0.001*** with respect to control statical analysis of the data is done via Anova. Values are considered significant when p-values< 0.05*

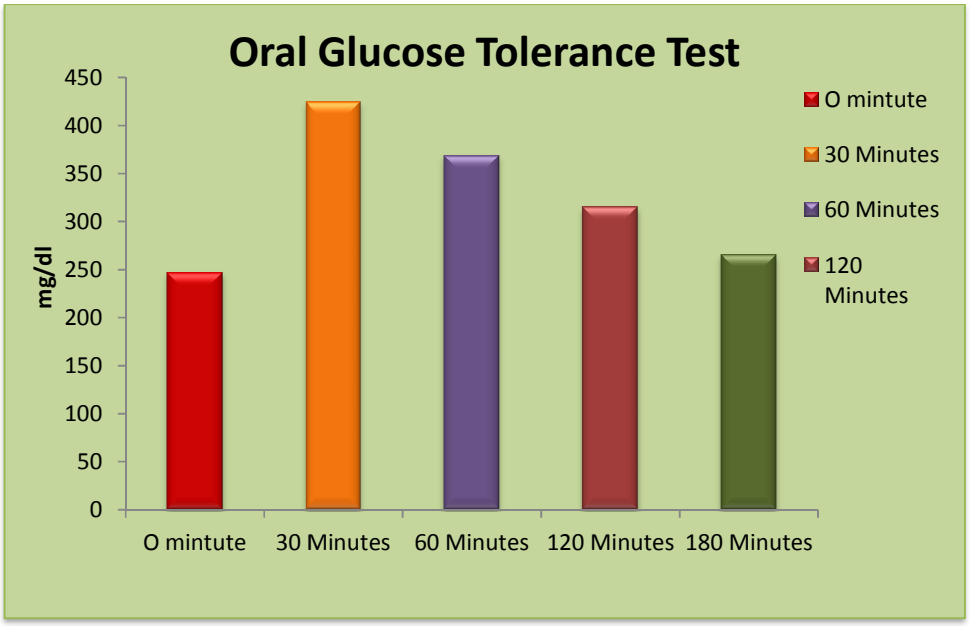

Fig. 1. OGTT of type II in STZ mediated diabetic model of rats

Table 2. Glibenclamide effect on blood glucose in rats

Groups	Experimental Design	Mean ± SEM
Group A	Control: Distilled water	126.6 ± 3.21
Group B	Positive Control: Streptozotocin	432.08±10.654***
Group C	Standard Group: Glibenclamide	108.50±2.14 ***

Values are shown as mean along with standard error of the mean (mean ± SEM) n= each set comprising 12 rats. Level of significance p < 0.05, p < 0.01**, p < 0.001*** with respect to control statical analysis of the data is done via Anova. Values are considered significant when p-values< 0.05*

Table 3. *Grewia asiatica* effect on rats blood glucose

Groups	Experimental Design	Mean ± SEM
Group A	Control: Distilled water	175 ± 3.07
Group B	Positive Control: Streptozotocin	395.145 ± 9.372
Group C	Standard Group: *Grewia asiatica*	115.0 ± 2.67***

value was 108.50 ± 2.14** with a comparison of control group 126.6 ± 3.21 presented in Table 4 Fig. 2, confirmed that both treated and standard group demonstrated significant outcomes.

The Streptozotocin mediated diabetes group blood glucose level was 198.30 ± 14.423***presented in Table 4 Fig. 2, demonstrated a significant result.

Table 4. Correlation of blood glucose level between control, diabetic, standard group and treated group

Groups	Experimental design	Mean ± SEM
Group A	Control: Distilled water	126.6 ± 3.21
Group B	Positive Control : Streptozotocin	198.30 ± 14.423 ***
Group C	Standard Group: Glibenclamide	108.50±2.14 ***
Group D	Treated Group: *Grewia asiatica*	115.0 ± 2.67 **

Values are shown as mean along with standard error of the mean (mean ± SEM) n= each set comprising 12 rats. Level of significance p < 0.05, p < 0.01**, p < 0.001*** with respect to control statical analysis of the data is done via Anova. Values are considered significant when p-values< 0.05*

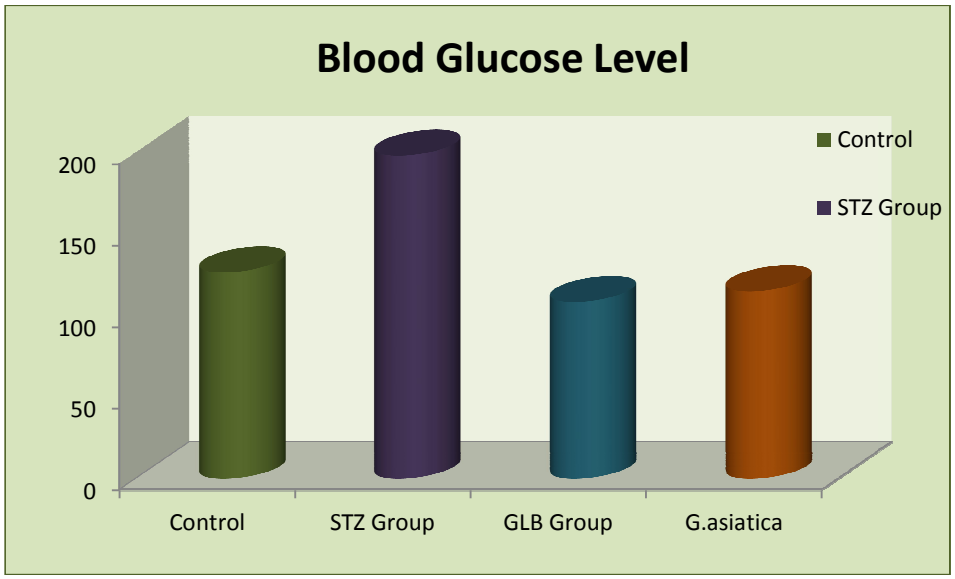

Fig. 2. Correlation of blood glucose level between control, STZ mediated diabetic, GLB, and *G. asiatica* group

4. DISCUSSION

Diabetes is a metabolic disorder and one of the prevailing diseases globally that causes the disturbance in glucose, proteins and fats metabolism [27] identifiedspecifically by glycosuria, hyperglycemia, polyuria and an overall decrease in weight [28]. Prevalence of DM globally in 1995 was estimated to be 4.00% which can rise to 5.40% by 2025. It is found to be greater in developed rather in developing nations [29]. In Asian states, more than 99.0% population is suffering from type-II diabetes. The prevalence and reverberation of type II diabetes in children are also escalating with its complications. Hyperglycemia is the most important parameter for the diagnosis of diabetes where the cause is an inability of insulin secretion and its activity or both. Hyperglycemia may lead to some serious complications i.e., vital organs damage like liver, kidneys, eyes, heart, and blood vessels. Chodury and his co-workers in 2002 revealed that the occurrence of micro and macrovascular diseases is less common in Europeans as compared to Asian patients [30]. In the 2012 report, IDF revealed that the incidence of diabetes will increases up to 565 million by 2030globally i.e., is one of the wake-up call [31].

Globally, the use of herbal products in TMS i.e., traditional medical system has a beneficial influence against various diseases especially on diabetes mellitus [32].

In 2009, Jabeen worked on 245 valuable plants of 77 families of trees (55), herbs (105), shrubs (54), grasses (10), a climber (15), and crops (6) and examined that 65% plants procure therapeutic effects. This information is supposed to serve as a reference for the assistance of health workers and the public in upcoming years [19].

In 2011, Marwat worked on the medicinal effects and importance of herbal flora and analyzed that *G. asiatica* possess various therapeutic properties[33]. Many types of research have proved various therapeutic and pharmacological effects from different parts of *G. asiatica*, such as properties against microbes, cancer, and anti-platelet effects have been observed upon studies on its leaves whereas its fruit possesses Ethno-botanical properties.[34] The main use of fruit is associated with its properties such as Stomachic, Anti-tumor, Anti-cancer, Astringent whereas the unripe form is usually used for its Anti-inflammatory, and Anti-pyretic activities.[35]It is also used in respiratory tract disorders, and cardiac as well as blood disorders.[21]

5. CONCLUSION

The observed results provide a much convincing affirmation regarding the *G. Asiatica* fruit, that it possesses hypoglycemic activity in mammalian model Albino Rats. Even so, the therapeutic effects of herbals in comparison to the alternative

medicines need a further assessment through a well-designed trial. However, standard preparations of herbs are essentially needed for further analysis and therapy productions. Conventional herbal medications used for the management of diabetes mellitus are thought for possible adverse herbal medications interactions also.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author from Research Unit at Baqai Institute of Pharmaceutical Sciences, Baqai Medical University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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