

Original Article

Anti-hyperglycemic and anti-hyperlipidemic effects of a methanolic extract of *Debregeasia salicifolia* in Alloxan-induced diabetic albino mice

Efeitos anti-hiperglicêmicos e anti-hiperlipidêmicos de um extrato metanólico de *Debregeasia salicifolia* em camundongos albinos diabéticos induzidos por aloxana

B. Khan^a, A. Ullah^b, M.A. Khan^c, A. Amin^d, M. Iqbal^e, S. Khan^f, M. Ateeq^g, K. Aman^b, A. Aziz^h, M. N. K. Khattak^b, T. Nadeemⁱ, N. Munir^j, S. Khan^k and Q. Ali^l 

^aDalian Medical University Liaoning, Department of Physiology, Dalian, China

^bDepartment of Health and Biological Sciences, Abasyn University Peshawar, Khyber Pakhtunkhwa Pakistan

^cHong Kong University of Science and Technology, Division of Life Science, Center for Cancer Research and State Key Lab for Molecular Neuroscience, Clear Water Bay, China

^dHong Kong Baptist University, School of Chinese Medicine and Department of Biology, Hong Kong, China

^eDepartment of Botany, Shaheed Benazir Bhutto Women university Peshawar, Khyber Pakhtunkhwa, Pakistan.

^fInstitute of microbiology and biotechnology, Bacha Khan University Charsada,

^gInstitute of biological sciences, Sarhad university of science and information technology Peshawar, Khyber Pakhtunkhwa Pakistan

^hUniversity of Sharjah, Department of Applied Biology, College of Sciences, Sharjah, United Arab Emirates.

ⁱUniversity of the Punjab, Centre of Excellence in Molecular Biology, Lahore, Punjab, Pakistan

^jCenter of biotechnology and microbiology, University of Peshawar, Khyber Pakhtunkhwa Pakistan

^kDepartment of biotechnology, university of swabi, Khyber Pakhtunkhwa Pakistan

^lThe University of Lahore, Institute of Molecular Biology and Biotechnology, Lahore, Punjab, Pakistan

Abstract

Diabetes mellitus (DM), an endocrine syndrome characterized by high blood glucose levels due to abrogated insulin activity. The existing treatments for DM have side effects and varying degrees of efficacy. Therefore, it is paramount that novel approaches be developed to enhance the management of DM. Therapeutic plants have been accredited as having comparatively high efficacy with fewer adverse effects. The current study aims to elucidate the phytochemical profile, anti-hyperlipidemic, and anti-diabetic effects of methanolic extract *D. salicifolia* (leaves) in Alloxan-induced diabetic mice. Alloxan was injected intraperitoneally (150 mg kg⁻¹, b.w), to induced diabetes in mice. The mice were divided into three groups (n=10). Group 1 (normal control) received normal food and purified water, Group II (diabetic control) received regular feed and clean water and group III (diabetic treated) received a methanolic extract of the plant (300 mg kg⁻¹) for 28 days with a typical diet and clean water throughout the experiment. Blood samples were collected to checked serum glucose and concentration of LDL, TC, TG. The extract demonstrated significant antihyperglycemic activity (P<0.05), whereas improvements in mice's body weight and lipid profiles were observed after treatment with the extract. This study establishes that the extract has high efficacy with comparatively less toxicity that can be used for DM management.

Keywords: diabetes mellitus, *debregeasia salicifolia*, hypoglycemic, hyperglycemic, hyperlipidemic activity, antidiabetic, phytochemicals, insulin, cholesterol.

Resumo

Diabetes mellitus (DM) é uma síndrome endócrina caracterizada por níveis elevados de glicose no sangue devido à atividade anulada da insulina. Os tratamentos existentes para o DM têm efeitos colaterais e vários graus de eficácia. Portanto, é fundamental que novas abordagens sejam desenvolvidas para aprimorar o manejo do DM. As plantas terapêuticas foram acreditadas como tendo eficácia comparativamente alta com menos efeitos adversos. O presente estudo visa elucidar o perfil fitoquímico, efeitos anti-hiperlipidêmicos e antidiabéticos do extrato metanólico de *D. salicifolia* (folhas) em camundongos diabéticos induzidos por aloxana. Alloxan foi injetado por via intraperitoneal (150 mg kg⁻¹, b.w), para induzir diabetes em camundongos. Os camundongos foram divididos em três grupos (n = 10). Grupo 1 (controle normal) recebeu ração normal e água purificada, Grupo II (controle diabético) recebeu ração regular e água limpa, e o grupo III (tratamento diabético) recebeu extrato metanólico da planta (300 mg kg⁻¹) por 28 dias com uma dieta típica e água limpa durante todo o experimento. Amostras

*e-mail: aminbiotech7@gmail.com; saim1692@gmail.com

Received: April 14, 2021 – Accepted: August 20, 2021



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

de sangue foram coletadas para verificar a glicose sérica e a concentração de LDL, TC, TG. O extrato demonstrou atividade anti-hiperglicêmica significativa ($P < 0,05$), enquanto melhorias no peso corporal e no perfil lipídico dos camundongos foram observadas após o tratamento com o extrato. Este estudo estabelece que o extrato tem alta eficácia com comparativamente menos toxicidade e pode ser usado para o controle do DM.

Palavras-chave: diabetes mellitus, *debregesia salicifolia*, atividade hipoglicêmica, hiperglicêmica e hiperlipidêmica, antidiabético, fitoquímicos, insulina, colesterol.

1. Introduction

Diabetes is an endocrine disease characterized by long-lasting high blood glucose levels primarily arising due to irregularities in insulin secretion and activity (Deepthi et al., 2017). The incidence of DM is increasing globally. Specialists predict that the number of people affected by diabetes is projected to increase 64% by 2025, meaning that 53.1 million people will be affected (Rowley and Bezold, 2012). The disorder has numerous pathogenic progressions ranging from autoimmune devastation of pancreatic β -cell and subsequent incomplete insulin scarcity (Type I) (Khazeem., 2011) or a decline in insulin production and activity (Type II). Prolonged hyperglycemia leads to problems in the breakdown of carbohydrates, fats and proteins causing extensive microvascular and macrovascular difficulties (Edem et al., 2021; Piero et al., 2012; Singh, et al., 2016) Other abnormalities include retinopathy with possible vision loss, nephropathy culminating in renal damage, ulcers, weakness of joints, autonomic loss of neurons associated with intestinal, urinary, vascular and sexual abnormalities (Piero et al., 2012). Diabetes patients commonly experience high blood glucose levels, frequent urination (polyuria), thirstiness (polydipsia), continuous appetite, loss in weight, visual impairment, and fatigue.

Modern treatments for DM can be limited by the costs of treatment, accessibility and varying side effects (Ali et al., 2021; Murugi et al., 2012; Mahmood et al., 2021). Glucose-lowering medicines like sulfonylureas, biguanides and insulin have also been linked to increased body weight and hypoglycemia (Mukundi et al., 2015). Therefore, the provision of effective medicines that have low associated side effects remain to be challenge (Shetti et al., 2012). Herbal remedies and traditional approaches are getting significant consideration from modern medicine practitioners, worldwide medicinal exploration and training organizations (Arif et al., 2021; Ashfaq et al., 2021; ALI et al., 2021; Siddique et al., 2021). The World Health Organization estimates that 80% of individuals in developing nations particularly Africa, use traditional medicine (Musila et al., 2002). Financial limitations have led many countries to search for low-priced management and treatment choices (Piero et al., 2012).

Plant-based herbal drugs offer a countless variety of bioactive constituents that can be used for the treatment of DM (Mahmood et al., 2012). The glucose-lowering agent, Metformin was initially a derivative of an old therapeutic herb *Galega officinalis*, which is still used by several communities as a treatment for diabetes. Certain plants have been verified to help and contribute to reducing minor difficulties associated with diabetes, while many others have been attributed to benefit the renewal of β -cells

and countering insulin opposition (Pandey et al., 2011). *Poppea capensis* and *Pterocarpus marsupium* have been used extensively in the past to manage DM (Karau et al., 2012). Studies have shown that the daily consumption of a 200 mg kg^{-1} ginseng extract decreases glucose levels and reduces fatigue in type II diabetes patients. A *Ginkgo biloba* extract has also been shown to be beneficial for managing first level diabetic neuropathy. At present herbal medications or their compounds are recommended broadly, even when their organic active constituents are unidentified (Middleton Junior et al., 2000). The World Health Organization (WHO) supports the consumption of herbal remedies for managing illnesses such as diabetes mellitus, and currently approximately more than 400 plants are being used for the management of DM. Therefore, there is rising interest towards the use of herbal medicines due to their efficiency, negligible side effects and comparatively lower costs.

The plant *Debregesea salicifolia* is commonly used plant in traditional treatment and having anti-bacterial activity (El-Mahmood et al., 2008; Shetti et al. 2012). The phytochemical screening of the *D. salicifolia* extract show the existence of various types of active components like Alkaloids, Tannins, aponins, Flavonoids, Anthraquinones, anthraquinones and tannins. Due to the presence of these active chemical compounds the plant has potential pharmacognostic significance (El-Mahmood et al., 2008). These compounds have been shown as being effective in the prevention and treatment of gastrointestinal diseases and cancers (El-Mahmood et al., 2008). Extracts of this plant are used for treating intestinal disorders such as diarrhea and dysentery, which are associated with bacterial infections (Akinpelu and Onakoya, 2006). Similarly, flavonoids are also used to treat various ailments. Previous *in vitro* studies have demonstrated that triterpenes and flavonoids have antibacterial and anti-cancer properties (Havsteen, 2002; Min et al., 2000). *D. salicifolia* extracts also have Ursolic acid and oleanolic acid (Akbar and Malik, 2002). These organic substances are also described as having anti-cancer properties. Their activity on HCT15 cells has been investigated (Li et al., 2002). The present study aims to scientifically access the glucose and cholesterol-lowering effects of *D. Salicifolia* extracts in Alloxan-induced diabetic albino mice.

2. Experimental Methods

2.1. Area of study

This investigation was conducted at the Department of Zoology, Hazara University Mansehra and National Veterinary Laboratory Islamabad, Pakistan.

2.2. Plant Material

Plant ingredients (leaves) were picked from their natural habitats in the Hazara division, Khyber Pakhtunkhwa, and were identified in the Department of Botany, Hazara University Mansehra. Vouchers specimens were also deposited; specimen number (BN/S1/5-001). Renewed foliage was washed away with purified water, dried out at normal room temperature (25°C) for eight days, away from direct sunlight, and crushed into a powder form with an electrical grinder. The crushed constituents were preserved at room temperature in dry plastic air-tight bags awaiting extraction.

2.3. Chemicals

To induce diabetes in mice, Alloxan was obtained from Sigma Aldrich Co., USA, and melted in phosphate buffered saline (PBS) solution. Methanol was used to extract the components of the plant whereas saline was used to soften it. Glucometer (Oncall) was used to check the blood glucose levels of mice.

2.4. Methanolic Extract preparation

Pulverized ingredients were completely dissolved in methanol by shaking the mixture for 72 hours using a shaker. The mixture was sieved with a piece of hygienic muslin fabric and then clarified by Whatman filter paper into a 1000 mL beaker. The acquired deposit was next discharged into the Rota vapor machine and kept stable at 50°C to vaporize the methanol. The remains were stored in glass bottle. Lastly, the concentrated portion of the methanolic extract was transferred into bottles and placed in a water bath at 40°C to vaporize the methanol totally. The final extracts were kept at room temperature (RT) until practice.

2.5. Experimental Animals

Thirty (30) healthy male BALB/C mice weighing 30–40 g with a mean weight of 35 g, were procured from the animal division of the National Institute of Health (NIH) in Islamabad and were carried to the National veterinary laboratory (NVL) in Islamabad. Hygienic polypropylene cages were used to house the mice in an investigational room with 12 hours of light contact. The mice were allowed to acclimatize to the new workroom for a week before the investigation. Normal nutrition was delivered containing pellet and clean water *ad libitum* throughout the investigation. The ethical guidelines used were the Animal Ethics Team Rules of the National Veterinary laboratory Islamabad and were strictly complied with during the investigation.

2.6. Induction of hyperglycemia

The mice were retained and starved overnight for twelve hours, they were then weighed, and the blood glucose level of all starved mice were tested and documented. The mice were starved overnight, and a single dose of newly prepared Alloxan in Phosphate Buffer Sulphate (PBS) was injected intraperitoneally (150 mg/Kg, b.w) (Hilaly et al., 2002). After treating with a dosage of Alloxan for three days, the

mice were deprived of food once more for 12 hours, their blood glucose was checked by (On-Call Extra) glucometer and their weight was also documented. Mice with blood glucose levels >200 mg/dl were measured as diabetic and were encompassed in the trial. Those mice without hyperglycemia after three days of Alloxan inoculation were excluded from the study.

2.7. Measurement of body weight

The bodyweight of the animals was tabulated six times during the study (i.e., before Alloxan injection (baseline values), 3 days after of Alloxan inoculation, first week, second week, third week and fourth week of the management time), with an ordinal weighing scale throughout the 28 days trial time and the changes in the body weight were noted.

3. Experimental Design

In our study, a total of 30 male mice were used and assigned randomly into three experimental groups, each group comprised of ten (10) mice.

Group I. The control group provided normal food and purified water *ad libitum* during the trial time.

Group II. The diabetic control group was fed a regular mouse diet and clean water *ad libitum* throughout the experiments.

Group III. This diabetic group of mice was treated with the plant extract at a dosage of 300 mg kg⁻¹ and given a typical diet and clean water *ad libitum* throughout the experiment.

The weight of all the mice in each group was documented up to the end of the experiments.

3.1. Administration of Solvent Fraction to diabetic group mice

The plant extract was prepared in distilled water and a single dose of 300 mg/kg of body weight was administered orally to the group of mice with diabetes once every 24 hours.

3.2. Blood collection and Glucose measurement

Blood samples were collected from the tail by cutting with a surgical blade. Blood glucose levels were measured using a glucometer and documented. The tail was cleaned with 70% ethanol after taking a sample.

3.3. Lipid profile determination

Mice were anesthetized before sacrificing and blood samples were taken directly from the heart using a disinfected syringe. Samples were kept standing until the plasma and serum were separated from the blood. The samples were then centrifuged at 5000 rpm for 15 min and the serum was stored at 4 until the lipid profile was tested. The serum concentration of high-density lipoprotein (HDL), low-density lipoprotein (LDL) were measured using a method as described (Friedewald et al., 1972). Total cholesterol (TC), and triglycerides (TG) was measured using a method as developed before (Trinder, 1969).

3.4. Qualitative phytochemical screening

The phytochemical screening was carried out for the presence or absences of secondary metabolites such as tannins, saponins, tormentic acid, alkaloids, terpenoids, pomolic acid, flavonoids, ursolic acid, cardiac glycosides.

3.5. Test for Tannins (Braemer's Test)

Extract of plant (0.25g) was put in 10 ml distilled water and boiled. The solution was filtered with filter paper (Wathman No. 1) in a test tube and then Ferric Chloride (FeCl) (0.1%) was added to the filtrate. The filtrate was observed, and the appearance of brownish green color shows the presence of Tannins.

3.6. Detection of saponins (Froth test)

The 0.25 g of plant extract was dissolved in 5 mL boiling distilled water in test tube and cool down. The solution was vigorously shaken for two minutes and the appearance of froth indicate that saponins were present in the plant extract.

3.7. Alkaloids Detection (Wagner's Test)

To the water dissolved 10 mg of plant extract 3 drops of Wagner's reagent was added and the appearance of reddish-brown color shows the presence of alkaloids.

3.8. Test for Terpenoids (Salkowski's test)

100 mg of 80% dried methanolic plant extract was dissolved in 5 mL distilled water followed by mixing in 2 mL chloroform. Then carefully 3 mL concentrated sulphuric acid (H₂SO₄) was added and appearance of reddish-brown color at interface confirms the terpenoids presence in the plant extract.

3.9. Test for Flavonoids

For the removal of fatty materials 0.5 g plant extract was shaken with pet ether and then dissolved in 20 mL of ethanol (80%). The solution is filtered, and following tests were performed with the filtrate for the presence of Flavonoids. a) Mixing of the filtrate (3 mL) with 1% AlCl₃ (4 mL) in MeOH. The appearance of yellow color indicates that flavonols, flavones are present in the extract. b) Mixing of filtrate (3 mL) with 1% KoH (4 mL) and appearance if dark yellow color confirms the presence of flavonoids.

3.10. Detection of anthraquinones (Borntrager's test)

The plant extract (1 g) was boiled with 1% HCl (6 mL) in test tube and filtered and then shake with benzene (5 mL). After removal of benzene 10% ammonium Hydroxide (NaOH) was added and appearance of pink, violet or red ring confirms anthraquinones.

3.11. Test for Coumarins

0.5 g of plant extract was put in a test tube having water and then covered with moistened 1N NaOH filter paper and suspend in boiling water for few minutes. The filter

paper was observed in UV light. Appearance of yellow florescence confirms the coumarins appearance.

3.12. Detection of Glycosides (Keller-kiliani test)

For the detection of Glycosides 0.5 g plant extract was taken in a test tube and 20 mL distilled water was added. After 24hr, filtration was done using filter paper and 5mL extract was treated with concentrated glacial acetic acid (2 mL) and few drops of FeCl₃ (0.1%). Then the whole mixture was put in 1mL concentrated H₂SO₄ test tube and appearance of brown ring at interface was observed.

3.13. Statistical analysis

The documented data were put in an Excel spreadsheet and analyzed statistically using GraphPad prism software (version 5). Outcomes were shown as Mean ± SD. Oneway Analysis of variance (ANOVA) test monitored by Tukey test for significant were utilized for numerous relationships among non-diabetic control mice group, the mice with diabetes and those treated with the plant extracts (300 mg kg⁻¹b.w). p<0.05 was considered statistically significant.

4. Results

4.1. Preliminary Phytochemical Screening

Phytochemical screening of *D. Salicifolia* shows the presence of some secondary metabolites summarized in the Table 1.

4.2. Effects of an orally administered *D. Salicifolia* extract on the levels of glucose in diabetes-induced mice.

The results show that *D. Salicifolia* extract treatment significantly (P<0.05) reduced the glucose concentration of the diabetes-induced mice treated with a dosage of 300 mg kg⁻¹ directed orally for 28 days in comparison to the diabetic control group. The glucose level in the diabetic group was found to have increased steadily from the beginning to the end of the experiment (315.8± 137.95 to 367.4±121.17), whereas the mice in the non-diabetic control groups had a relatively stable level (118.8±19.98 to 120.3±7.89). The initial high levels of glucose were found to have been significantly (P<0.05) reduced in the treatment group with the constant provision of *D Salicifolia* extracts over a period of 28 days when compared to the diabetic control group (Table 2)

Table 1. Phytochemical Constituents of *D. Salicifolia*.

Metabolites	Name of Test	Results
Tannins	Braemer's Test	++
saponins	Froth test	++
Alkaloids	Wagner's Test	++
Terpenoids	Salkowski's test	++
Flavonoids	NaOH/KOH Test	++
Coumarins		--
Glycosides	Keller-kiliani test)	--

Notes: ++ = Test Positive; -- = Test Negative.

4.3. Measurements of Lipid profile

The current investigation showed the importance of elevated cholesterol levels in diabetes-induced mice. The whole cholesterol (TG, HDL, LDL, and TC) levels were found to be highly elevated in the diabetic group. Elevated level of high-density lipoprotein (HDL) was relatively less in the diabetic group, which shows the dangerous implications for the development of vascular diseases. The quantity of total cholesterol, triglycerides and LDL were investigated and found to be reduced after treatment with the extract even though the reduction was only marginal. The amount of high-density lipoprotein (HDL) and others were found to be normal in the extract-treated group. This suggests that the *D. salicifolia* extract is effective in normalizing cholesterol levels in mice. In our findings, total cholesterol, triglycerides, LDL and HDL were somewhat changed in the serum of the control and treatment groups as shown in (Table 3).

4.4. Body Weight changes in mice after treatment with extract

The bodyweight of the control and treated mice were found to be increasing over time as opposed to the decrease seen in the diabetic mice, potentially due to alloxan treatment. In this present study, the oral administration of

D. Salicifolia extract at a concentration of 300 mg kg⁻¹b.w, was found to increase the weight of the mice significantly ($P < 0.05$) when compared to the diabetic control mice and baseline weight of the animals. This improvement in body weight we propose it due to the influence of the extract of *D. Salicifolia* over a period of 28 days. Body weights were measured five times during the study period (i.e., Baseline [before injection of Alloxan] as day 1, day 7, day 14, day 21, day 28 of the study period), with a digital scale from NVL Islamabad. The current result shows that those mice that were treated with the extract began showing an elevation in body size which may be attributed to protein build-up in the body. This influence of the extract could be due to antihyperglycemic action leading to the recovery of the body weight. Variation in the body weight throughout the entire study over time is shown in (Table 4).

5. Discussion

Diabetes is an endocrine disorder associated with high blood glucose levels and irregular metabolism. Long-lasting high levels of glucose are related to numerous problems, such as cardiovascular disease, kidney disease and visual defects (SINGH et al., 2016) Existing treatments for diabetes have numerous adversities (Mukundi et al., 2015). Therefore,

Table 2. Effect of *D. salicifolia* plant extract on fasting blood glucose levels Alloxan-induced diabetic albino mice after four weeks of treatment.

Treated groups	Fasting BG level (mg/dl)				
	Initial valve	After one week	After two weeks	After three weeks	After four weeks
Normal Control	118.8±19.98	93.9±11.37	109.5±13.31	106.8±14.61	120.3±7.89
Diabetic Control	315.8± 137.95	322.2±102.86	334.7±78.49	346.4±86.43	367.4±121.17
<i>D.salicifolia</i> extract300 mg kg ⁻¹	296.9±135.5	247.5±115.14*	198.6±91.02*	176.4±76.84*	154.3±63.97*

* $P < 0.05$. Data represents Means ± SD, (n=10).

Table 3. Effect of *D salicifolia* plant extract on Lipid profile of Alloxan-induced diabetic albino mice after four weeks of treatment.

Treatment Groups	Lipid profile			
	(TG)	(HDL)	(LDL)	(TC)
Normal control	75.67±13.32	43±3.00	55.67±3.00	114.33±12.10
Diabetic Control	117.33±23.86	35.67±3.51	88.33±10.69	141.33±8.74
Extracted treated300 mg kg ⁻¹	100.33±17.01	38.67±3.51	83±1.00	138.67±30.43

Data represents Means ± SD, (n=10).

Table 4. Effect of *Debregeasia salicifolia* plant extract on body weight of Alloxan-induced. Diabetic albino mice after four weeks of treatment.

Treatment groups	Bodyweight (gm.)				
	1 st day	7 th day	14 th day	21 st day	28 th day
Normal Control group	34±2.11	34±2.11	33±2.58	36.5±2.42	35±2.36\
Diabetic Control	36±2.48	334.5±2.84	31.3±3.31	28.1±2.57	25±2.32
<i>D. salicifolia</i> extract 300 mg kg ⁻¹	34±2.78	32.3±3.89	32.8±81.02*	33.5±3.70*	34.5±2.57*

* $P < 0.05$. Data showed Means ± SD, (n=10).

the exploration of safe, accessible and low-priced anti-diabetic substances needs to be sustained (Rajagopal and Sasikala, 2008).

Plant derivatives have been demonstrated as being active and effective in treating DM. The WHO estimates that 80% of people worldwide utilize plant remedies for the treatment of different types of diseases (Alarcon-Aguilar et al., 2000). The consumption of plant extracts and their active compounds has increased worldwide owing to the evident benefits. In this investigation, diabetes was induced by the administration of an inducing agent called Alloxan monohydrate. This drug destroys and decreases the pancreatic β -cell population in the islets of Langerhans by the production of reactive oxygen species i.e. nitric oxide (Szkudelski, 2001). The oral administration of a methanolic leaf extract of *D. salicifolia* in diabetic mice revealed glucose-lowering activities, indicating that the plants constituents have glucose-lowering compounds. The blood glucose reducing activity of the extract may be due to an increase in the use of glucose by marginal cells in muscles, liver and fat cells owing to increased insulin sensitivity and upregulation of insulin receptors or short activation of β -cells of the pancreas lead to insulin release (Ayodhya et al., 2010). The anti-hyperglycemic activity of the extract may also be due the interference of nutritive carbohydrates and disaccharide absorption in the small intestines of mice resulting in digestive flexibility and pouring (Esmaeili and Yazdanparast, 2004). This could also be due to renewed β -cells (Sharma et al., 2006; Lombardo and Chicco, 2006), and/or increased sensitivity to insulin. The extract of the plant may also enhance liver function such as the uptake of glucose, facilitating the transportation of serum glucose to outlying tissue and consumption (Ikmal et al. 2013). The glucose reducing influence of our plant extract is similar to that observed in previous studies. For example, the aqueous leaf extracts of *Helichrysum odoratissimum* showed anti-diabetic action by improving either the secretion of pancreatic insulin from the β -cells or the discharge of attached insulin (Njagi et al., 2015). A chemical component present in garlic has also been shown to exert anti-oxidative action by removing reactive oxygen species and enhancing cellular antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase (Njagi et al., 2015). Murugi et al., (2012) demonstrated that *Caesalpinia volkensii* leaf extracts show a glucose-lowering effect in Alloxan-induced diabetic mice at dosage of 50, 100 and 150 mg kg⁻¹ by weight the administration of *Momordica charantia* orally at a dosage of 300 mg kg⁻¹ body weight also reduced the fasting blood glucose levels in mice (Xu et al., 2015). There is a higher anti-diabetic effect observed when anti-hyperglycemic plant extracts are administered intraperitoneally as compared to the oral route, potentially due to the relatively reduced rate of assimilation (Meezan et al., 2005). The plant extract in our study may be absorbed by active transport at a dosage of 300 mg kg⁻¹ of body weight. The lesser glucose levels in mice orally and intraperitoneally administered with extracts can be a consequence of high glycolysis (Meezan et al., 2005). The methanolic leaf extract of *D. salicifolia* indicates insulin-mimetic action and at times worked better than traditional medicines orally administered, these may be

due to the element that increases absorption of glucose by marginal mediation of GLUT-4 or the extracts might be effortlessly immersed in the intraperitoneal cavity and gastrointestinal mucosa.

The anti-hyperglycemic influence of the extracts of the *D salicifolia* might also be accredited to the existence of numerous phytochemical ingredients it contains sterols, tannins, saponins, alkaloids, terpenoids, flavanoids, free and bound anthraquinones etc, that have been related with anti-diabetic influence (Modak et al., 2007). The conclusion of the anti-diabetic effects of *D. salicifolia* could be attributed therefore to these detected components. The polyhydroxylated flavonol improves lipid production and glucose absorption in the adipocyte's tissues, likewise, flavonoid and myricetin have insulin-mimetic activities (Modak et al., 2007). Epicatechin and its active ingredients have been shown to enable insulin discharge through the conversion of proinsulin to insulin *in vitro*. It has been revealed that the flavonoid component from *Pterocarpus marsupium* could be indicated for pancreatic beta-cell regeneration (Li et al., 2009). Flavonoid glycosides such as isostrictinin, pedunculagin, and strictinin are the active ingredients of *Psidium guajava*, which are being used in the clinical management of diabetes due to increased insulin secretion (Li et al., 2009) and this is also observed in our studied plant. At 50-150 mg kg⁻¹ flavonoids isolated from the leaf of *Ipomoea batatas* decreases lipid and glucose level in Alloxan-induced diabetic mice (Shukla et al., 2012) corroborating with the outcome of our study where blood glucose and lipid levels, were improved. The aqueous leaf extract of *Lippia javanica* consists of alkaloids, which are recognized to have blood glucose level lowering capacity, also found in the plant *D salicifolia* El-Mahmood et al., (2008). An alkaloid fraction from *C. decidua* exhibits hypoglycemic prospective in mice (Sharma et al., 2010), the same component was found in our studied extract.

Alkaloids and tetrandrine show antioxidant actions attributable to many natural actions related to this plant's anti-diabetic influence. The alkaloids l-ephedrine of *Ephedra distachya* plant has shown glucose-lowering action in mice with diabetes due to restoration and renewal of atrophied pancreatic cells that discharge insulin (Piero et al., 2015; Alarcon-Aguilar et al., 2000). Similar constituents were also investigated in the *D salicifolia* plant, which has confirmed glucose reducing effects. The aqueous leaf extract of *Lippia javanica* contains saponins that have been shown to exhibit glucose-lowering effects (Arika et al., 2015). The intraperitoneal dosage of 100, 200 mg kg⁻¹ b.w of the leaf extract of *Acanthopanax senticosus* in Alloxan-induced diabetic mice showed the presence of saponins (that are also present in *D salicifolia*) that lower blood glucose and adrenaline levels without effecting the blood glucose levels in untreated mice.

Kumari et al. confirmed that 50% of *Acacia nilotica* consists tannins which have been shown to reduce blood glucose in diabetic mice (Kumari et al., 2014). The methanolic leaf extract of *D salicifolia* also consists of tannins that are known to have anti-diabetic effects. In medical reports, all kinds of tannins may contribute to managing sugar concentration in blood. Tannin has been revealed to motivate the receptor tissues to utilize

glucose (Kumari et al., 2014). The methanolic leaf extracts of *D. salicifolia* also consists of terpenoids that have sugar-lowering constituents. Terpenoids have been revealed to lower diastolic blood pressure and decrease the glucose in the blood of hypertensive and diabetic patients correspondingly (Piero et al., 2015). Terpenoids also improve skin quality, increase the number of antioxidants in wounds, and renovate swollen tissues by improving blood circulation (Piero et al., 2015). The seeds and leaves of *S. spectabilis* are utilized for the management of diabetes mellitus due to the presence of terpenoids

Lipids play a crucial function in the etiology of diabetes (Sharma et al., 1996). The main problems of high lipid levels in diabetes are hypercholesterolemia and hypertriglyceridemia (Al-Shamaony et al., 1994). In the current study, it was discovered that the methanolic extract of *D. Salicifolia* decreases blood cholesterol levels at a dosage of 300 mg kg⁻¹ of b.w., although this was not found to be significant at this dosage (P>0.05).

Increased HDL levels were also observed, whereas a reduction was observed in the LDL values although not significant. Cholesterol levels in streptozotocin-induced diabetic rats decreased following *Nigella sativa* extract treatments at concentrations of (350 mg kg⁻¹) and *Cinnamomum zeylanicum* extract (500 mg kg⁻¹) for twenty-one days, compared to the diabetic control group (Al-Logmani, 2009). In the present work, Alloxan-induced diabetic mice were cured with *D. salicifolia* extract at a dose of 300 mg kg⁻¹ for 28 days which reduced the levels of low-density lipoprotein-cholesterol (LDL), increased high-density lipoprotein (HDL) and also normalized cholesterol (TC) and Triacylglycerol (TG) marginally (P>0.05). Though the amount of *D. salicifolia* was different from the doses of *N. sativa* and *C. zeylanicum* extracts the treatment dose and period need to be optimized in future investigations.

The *Momordica cymbalaria* extract displayed a significant (p<0.05) reduction in TC, TG, LDL, with an upsurge in HDL at a dosage of 250 mg kg⁻¹ for two weeks (Radhika et al., 2015), a result that agrees with our study in which 300 mg kg⁻¹ of *D. salicifolia* was administered. The enhanced hypolipidemic influence of *M. cymbalaria* extract may be due to the presence of different active compounds within the plant as it showed impressive results of lowering concentration and treatment time as compared to the *D. salicifolia* extract.

The reduced cholesterol levels following *D. Salicifolia* extract treatment may be due to the high efficiency of certain enzymes like lecithin cholesterol acyltransferase enzymes, which regulates blood lipid concentration and changes the fatty acid into its deposited form (triglyceride).

Chloroform leaf extracts of *Nerium Indicum* were given at a dose of 500 mg kg⁻¹ b.w for one week which significantly (P<0.05) improved the bodyweight of Alloxan-induced diabetic rats (Sikarwar et al., 2009). In the present study the methanolic extract of *D. salicifolia* was directed at an amount of 300 mg kg⁻¹ to Alloxan-induced diabetic mice for four weeks, which resulted in a significant (P<0.05) improvement in the bodyweight of mice. The dose of *D. salicifolia* was smaller than that of *N. indicum*, but it is noted that dose of 500 mg kg⁻¹ of *N. indicum* extract is much more effective than the 300 mg kg⁻¹ dose of *D.*

salicifolia extract in shorter periods for the treatment of DM. The blood glucose level reduction observed in this study was comparatively higher than that observed for other agents in previous studies.

6. Conclusion

The methanolic leaf extracts of *D. salicifolia* has anti-diabetic potential due to the existence of important phytochemicals that confer the anti-diabetic actions. The oral administration of methanolic extract was however found to be very active in lowering the blood glucose, normalizing weight as well as marginally improving lipid profiles. The anti-diabetic potential of the plant extract investigated may be due to the occurrence of phytochemicals. Therefore, this work proves the medicinal use of *D. salicifolia* and demonstrates its effectiveness in controlling DM. Further investigations need to be carried out focusing on the molecular underpinnings leading to its use in treating DM. Investigation and utilization of this plant in advanced wildlife or human subject experiments should also be planned to determine its full drug potential.

Acknowledgements

The authors wish to thank Dr. Imam Shah (Research Officer) at National Veterinary Laboratory Islamabad, Pakistan for allowing access to resources for experimental purpose and analysis.

References

- AKBAR, E. and MALIK, A., 2002. Antimicrobial triterpenes from *Debregeasia salicifolia*. *Natural Product Letters*, vol. 16, no. 5, pp. 339-344. <http://dx.doi.org/10.1080/10575630290033088>. PMID: 12434990.
- AKINPELU, D.A. and ONAKOYA, T.M., 2006. Antimicrobial activities of medicinal plants used in folklore remedies in south-western. *African Journal of Biotechnology*, vol. 5, pp. 1078-1081.
- ALARCON-AGUILAR, F.J., JIMENEZ-ESTRADA, M., REYES-CHILPA, R. and ROMAN-RAMOS, R., 2000. Hypoglycemic effect of extracts and fractions from *Psacalium decompositum* in healthy and alloxan-diabetic mice. *Journal of Ethnopharmacology*, vol. 72, no. 1-2, pp. 21-27. [http://dx.doi.org/10.1016/S0378-8741\(00\)00202-6](http://dx.doi.org/10.1016/S0378-8741(00)00202-6). PMID: 10967449.
- ALI, I., IDREES, N., SIDDIQUE, A., SHAH, K.H., AHMAD, R., ALI, A. and FATEH, A., 2021. Microbial composition of processed foods. *Biological and Clinical Sciences Research Journal*, vol. 2021, no. 1, pp. e025. <http://dx.doi.org/10.54112/bcsrj.v2021i1.74>.
- AL-LOGMANI, A.S., 2009. Effects of *Nigella sativa* L. and *cinnamomum zeylanicum* blume oils on some physiological parameters in streptozotocin-induced diabetic rats. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, vol. 8, pp. 86-96.
- AL-SHAMAONY, L., AL-KHAZRAJI, S.M. and TWAJJ, H.A., 1994. Hypoglycaemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. *Journal of Ethnopharmacology*, vol. 43, no. 3, pp. 167-171. [http://dx.doi.org/10.1016/0378-8741\(94\)90038-8](http://dx.doi.org/10.1016/0378-8741(94)90038-8). PMID: 7990489.

- ARIF, F., TAHIR, T., SUHAIL, S., ANEES, R., NADEEM, I. and HAFEEZ, M.M., 2021. Genetic factors associated with mutations of molecular mechanism and drug resistance in *Mycobacterium tuberculosis*. *Biological and Clinical Sciences Research Journal*, vol. 2021, no. 1, pp. e024. <http://dx.doi.org/10.54112/bcsrj.v2021i1.72>.
- ARIKA, W.M., OGOLA, P.E., ABDIRAHMAN, Y.A., MAWIA, A.M., WAMBUA, F.K., NYAMAI, D.W., KIBOI, N.G., WAMBANI, J.R., NJAGI, S.M., RACHUONYO, H.O., MUCHORI, A.N., LAGAT, R.C., AGYIRIFO, D.S., NGUGI, M.P. and NJAGI, E.N.M., 2015. In Vivo safety of aqueous leaf extract of *lippia javanica* in mice models. *Biochemistry and Physiology*, vol. 5, pp: 2.
- ASHFAQ, F., Q. ALI, M.A. HAIDER, M.M. HAFEEZ, and A. MALIK, 2021. Therapeutic activities of garlic constituent phytochemicals. *Biological and Clinical Sciences Research Journal*, vol. 2021, no. 1, pp. 53.
- AYODHYA, S., KUSUM, S. and ANJALI S., 2010. Hypoglycaemic activity of different extracts of various herbal plants. *International Journal of Research in Ayurveda and Pharmacy*, vol. 1, no. 1, pp. 212-224.
- DEEPTHI, B., SOWJANYA, K., LIDIYA, B., BHARGAVI, R.S. and BABU, P.S., 2017. A modern review on Diabetes mellitus: an inhibitory metabolic disorder. *Journal in silico & In Vitro Pharmacology*, vol. 3, pp. 1-14.
- EDEM, G.D., OKON, K.A., ESSIEN, S.I. and BASSEY, E.I., 2021. Lantana camara: a potent influential factor in improving the gastric mucosa of wistar rats ravaged by ulcer. *Biological and Clinical Sciences Research Journal*, vol. 2021, no. 1, pp: e017.
- EL-MAHMOOD, A.M., J.H. DOUGHARI, and N. LADAN, 2008. Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology*, vol. 2, pp: 089-094.
- ESMAEILI, M.A. and YAZDANPARAST, R., 2004. Hypoglycaemic effect of *Teucrium polium*: studies with rat pancreatic islets. *Journal of Ethnopharmacology*, vol. 95, no. 1, pp. 27-30. <http://dx.doi.org/10.1016/j.jep.2004.06.023>. PMID:15374603.
- FRIEDEWALD, W.T., LEVY, R.I. and FREDRICKSON, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, vol. 18, no. 6, pp. 499-502. <http://dx.doi.org/10.1093/clinchem/18.6.499>. PMID:4337382.
- HAVSTEEN, B.H., 2002. The biochemistry and medical significance of the flavonoids. *Pharmacology & Therapeutics*, vol. 96, no. 2-3, pp. 67-202. [http://dx.doi.org/10.1016/S0163-7258\(02\)00298-X](http://dx.doi.org/10.1016/S0163-7258(02)00298-X). PMID:12453566.
- HILALY, J. and LYOUSSI, B., 2002. Hypoglycaemic effect of the lyophilised aqueous extract of *Ajuga iva* in normal and streptozotocin diabetic rats. *Journal of Ethnopharmacology*, vol. 80, no. 2-3, pp. 109-113. [http://dx.doi.org/10.1016/S0378-8741\(01\)00407-X](http://dx.doi.org/10.1016/S0378-8741(01)00407-X). PMID:12007699.
- IKMAL, S.I.Q.S., HURI, H.Z., VETHAKKAN, S.R. and AHMAD, W.A.W., 2013. Potential biomarkers of insulin resistance and atherosclerosis in type 2 diabetes mellitus patients with coronary artery disease. *International Journal of Endocrinology*, vol. 2013, pp. 698567. PMID:24282409.
- KARAU, G.H., NJAGI, E.N.M., MACHOCHO, A.K., WANGAI, L.N. and KAMAU, P.N., 2012. Hypoglycemic activity of aqueous and ethylacetate leaf and stem bark extracts of "*Pappea capensis*" in alloxan-induced diabetic BALB/c mice. *British Journal of Pharmacology and Toxicology*, vol. 3, pp. 251-258.
- KHAZEEM, M. M. 2011. *Study the effect of teucrium Polium L.Aerial Parts Extract on Normal and Alloxan-Induced Diabetic mice*. Iraq: College of Science, Al-Nahrain University. 101 p. M. Sc. Thesis.
- KUMARI, M., JAIN, S. and DAVE, R., 2014. Babul (*Acacia nilotica*): a potential source of tannin and its suitability in management of type II diabetes. *Nutrition & Food Science*, vol. 2, no. 2, pp. 122-124. <http://dx.doi.org/10.1108/NFS-06-2013-0072>.
- LI, F., LI, Q., GAO, D. and PENG, Y., 2009. The optimal extraction parameters and anti-diabetic activity of flavonoids from *Ipomoea batatas* leaf. *African Journal of Tradit Complement Altern Med*, vol. 6, no. 2, pp. 195-202. PMID:20209012.
- LI, J., GUO, W. and YANG, Q., 2002. Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. *World Journal of Gastroenterology*, vol. 8, no. 3, pp. 493-495. <http://dx.doi.org/10.3748/wjg.v8.i3.493>. PMID:12046077.
- LOMBARDO, Y.B. and CHICCO, A.G., 2006. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. *The Journal of Nutritional Biochemistry*, vol. 17, no. 1, pp. 1-13. <http://dx.doi.org/10.1016/j.jnutbio.2005.08.002>. PMID:16214332.
- MAHMOOD, A., MAHMOOD, A. and QURESHI, R.A., 2012. Antimicrobial activities of three species of family mimosaceae. *Pakistan Journal of Pharmaceutical Sciences*, vol. 25, no. 1, pp. 203-206. PMID:22186331.
- MAHMOOD, H., ALI, Q., HAFEEZ, M.M. and MALIK, A., 2021. Antioxidant activity of *Syzygium aromatum* and *Cinnamomum verum* seed extracts. *Biological and Clinical Sciences Research Journal*, vol. 2021, no. 1, pp. e015. <http://dx.doi.org/10.54112/bcsrj.v2021i1.63>.
- MEEZAN, E., MEEZAN, E.M., JONES, K., MOORE, R., BARNES, S. and PRASAIN, J.K., 2005. Contrasting effects of puerarin and daidzin on glucose homeostasis in mice. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 22, pp. 8760-8767. <http://dx.doi.org/10.1021/jf058105e>. PMID:16248582.
- MIDDLETON JUNIOR, E., KANDASWAMI, C. and THEOHARIDES, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews*, vol. 52, no. 4, pp. 673-751. PMID:11121513.
- MIN, B.S., KIM, Y.H., LEE, S.M., JUNG, H.J., LEE, J.S., NA, M.K., LEE, C.O., LEE, J.P. and BAE, K., 2000. Cytotoxic triterpenes from *Crataegus pinnatifida*. *Archives of Pharmacol Research*, vol. 23, no. 2, pp. 155-158. <http://dx.doi.org/10.1007/BF02975505>. PMID:10836742.
- MODAK, M., DIXIT, P., LONDHE, J., GHASKADBI, S. and DEVASAGAYAM, T.P.A., 2007. Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of Clinical Biochemistry and Nutrition*, vol. 40, no. 3, pp. 163-173. <http://dx.doi.org/10.3164/jcbn.40.163>. PMID:18398493.
- MUKUNDI, M.J., PIERO, N.M., MWANIKI, N.E.N., MURUGI, N.J., DANIEL, A.S., GATHUMBI, K.P. and MUCHUGI, N.A., 2015. Antidiabetic effects of aqueous leaf extracts of *acacia nilotica* in alloxan induced diabetic mice. *Journal of Diabetes & Metabolism*, vol. 6, no. 7, pp. 1000568. <http://dx.doi.org/10.4172/2155-6156.1000568>.
- MURUGI, N.J., PIERO, N.M., MWITI, K.C., JOSEPH, N.N.J., MWANIKI, N.E.N., WILSON, N.M., DAVID, M. and KARURI, G.P., 2012. Hypoglycemic effects of *Caesalpinia volkensii* on alloxan-induced diabetic mice. *Asian Journal of Pharmaceutical and Clinical Research*, vol. 5, suppl. 2, pp. 69-74.
- MUSILA, W., KISANGAU, D. and MUEMA, J., 2002. Conservation status and use of medicinal plants by traditional medical practitioners in Machakos District, Kenya. *National Museums of Kenya*, vol. 22, pp. 12-18.
- NJAGI, J.M., NGUGI, M.P., KIBITI, C.M., NGERANWA, J., NJUE, W., GATHUMBI, P. and NIAGI, F., 2015. Hypoglycemic effect of *Helichrysum odoratissimum* in alloxan induced diabetic mice. *Phytopharmacology*, vol. 4, pp. 30-33.

- PANDEY, A., TRIPATHI, P., PANDEY, R., SRIVATAVA, R. and GOSWAMI, S., 2011. Alternative therapies useful in the management of diabetes: A systematic review. *Journal of Pharmacy & Bioallied Sciences*, vol. 3, no. 4, pp. 504-512. <http://dx.doi.org/10.4103/0975-7406.90103>. PMID:22219583.
- PIERO, M.N., NJAGI, J.M., KIBITI, C.M., NGERANWA, J.N. and NJAGI, E.N.M., 2012. Metabolic complications of diabetes mellitus: a review. *South Asian Journal of Biological Sciences*, vol. 2, pp. 37-49.
- PIERO, N.M., KIMUNI, N.S., NGERANWA, N.J., ORINDA, O.G., NJAGI, M.J., MAINA, D., AGYIRIFO, S.D., GATHUMBI, K., KING'E, W.S. and NJAGI, E.N.M., 2015. Antidiabetic and safety of *Lantana rhodesiensis* in alloxan induced diabetic rats. *Journal of Developing Drugs*, vol. 4, pp. 1-10.
- RADHIKA, T., BAIRI, R. and MOUNIKA, G., 2015. Anti-obesity and anticoagulant activity of ethanol extract of *Trigonella foenum-graecum* L. *International Journal of Research in Pharmacy & Pharmacology*, vol. 4, pp. 119-125.
- RAJAGOPAL, K. and SASIKALA, K., 2008. Antihyperglycaemic and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats. *Singapore Medical Journal*, vol. 49, no. 2, pp. 137-141. PMID:18301841.
- ROWLEY, W.R. and BEZOLD, C., 2012. Creating public awareness: state 2025 diabetes forecasts. *Population Health Management*, vol. 15, no. 4, pp. 194-200. <http://dx.doi.org/10.1089/pop.2011.0053>. PMID:22283662.
- SHARMA, B., SALUNKE, R., BALOMAJUMDER, C., DANIEL, S. and ROY, P., 2010. Anti-diabetic potential of alkaloid rich fraction from *Capparis decidua* on diabetic mice. *Journal of Ethnopharmacology*, vol. 127, no. 2, pp. 457-462. <http://dx.doi.org/10.1016/j.jep.2009.10.013>. PMID:19837152.
- SHARMA, K., JIN, Y., GUO, J. and ZIYADEH, F.N., 1996. Neutralization of TGF- β by anti-TGF- β antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes*, vol. 45, no. 4, pp. 522-530. <http://dx.doi.org/10.2337/diab.45.4.522>. PMID:8603776.
- SHARMA, S.B., NASIR, A., PRABHU, K.M. and MURTHY, P.S., 2006. Antihyperglycemic effect of the fruit-pulp of *Eugenia jambolana* in experimental diabetes mellitus. *Journal of Ethnopharmacology*, vol. 104, no. 3, pp. 367-373. <http://dx.doi.org/10.1016/j.jep.2005.10.033>. PMID:16386863.
- SHETTI, A.A., SANAKAL, R.D. and KALIWAL, B.B., 2012. Antidiabetic effect of ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic mice. *Asian Journal of Plant Science Research*, vol. 2, pp. 11-15.
- SHUKLA, S., MEHTA, A., MEHTA, P. and BAJPAI, V.K., 2012. Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert. *Experimental and Toxicologic Pathology*, vol. 64, no. 7-8, pp. 807-811. <http://dx.doi.org/10.1016/j.etp.2011.02.002>. PMID:21377849.
- SIDDIQUE, A., IDREES, N., KASHIF, M., AHMAD, R., ALI, A., ALI, A., SIDDIQUA, A. and JAVIED, M.A., 2021. Antibacterial and antioxidant activity of Kiwi fruit. *Biological and Clinical Sciences Research Journal*, vol. 2021, no. 1, pp. e028. <http://dx.doi.org/10.54112/bcsrj.v2021i1.76>.
- SIKARWAR, M.S., PATIL, M.B., KOKATE, C.K., SHARMA, S. and BHAT, V., 2009. Antidiabetic activity of *Nerium indicum* leaf extract in alloxan-induced diabetic rats. *Journal of Young Pharmacists : JYP*, vol. 1, no. 4, pp. 330-335. <http://dx.doi.org/10.4103/0975-1483.59323>.
- SINGH, N., KESHERWANI, R., KUMAR, A. and DILIP, D.K., 2016. A review on diabetes mellitus. *The Pharma Innovation Journal*, vol. 5, no. 7, pp. 36-40.
- SZKUDELSKI, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, vol. 50, no. 6, pp. 537-546. PMID:11829314.
- TRINDER, P., 1969. Determination of serum cholesterol by enzymatic colorimetric method. *Annals of Clinical Biochemistry*, vol. 6, no. 24, pp. 7.
- XU, X., SHAN, B., LIAO, C.H., XIE, J.H., WEN, P.W. and SHI, J.Y., 2015. Anti-diabetic properties of *Momordica charantia* L. polysaccharide in alloxan-induced diabetic mice. *International Journal of Biological Macromolecules*, vol. 81, pp. 538-543. <http://dx.doi.org/10.1016/j.ijbiomac.2015.08.049>. PMID:26318666.