

Research Article

Antihyperglycemic effect of *Woodfordia fruticosa* (Linn) Kurz in STZ-induced diabetic rats**Mohammed Shakir Ghouse^{*1}, A.M.Danish²**¹Dr. Vedprakash Patil Pharmacy College, Aurangabad, Maharashtra, India²SDMVM's Diploma in Pharmacy Institute, Aurangabad, Maharashtra, India**Abstract**

The present study was carried out to investigate *Woodfordia fruticosa* Linn (Kurz) for pharmacological screening of flower extract of the plant in streptozotocin induced diabetic rats. *Woodfordia fruticosa* Linn (Kurz) was collected in the month of January, from kumta district. It was authenticated by renowned botanist. Authenticated plant material was evaluated morphologically and microscopically. Air dried powdered flower of the plant was extracted successively using alcohol as solvent and water extract by maceration process. Acute oral toxicity studies was carried out and two non lethal doses 100 and 200 mg/kg p.o was selected for the Antihyperglycemic activity. To confirm the anti-diabetic activity of the plant the OGTT were carried out in normal rats, followed by various biochemical parameters such as Serum insulin (SI), serum triglyceride (TG), Serum total cholesterol (TC), HDL-c, VLDL-c, LDL-c, TC/HDL-c. The WFA and WFE extracts (100 mg/kg) significantly reduced the SG levels, normalization of lipid profile and increased insulin levels in diabetic treated rats. Higher dose of WFE and WFA (200 mg/kg) was more effective than the lower dose and the results was comparable with diabetic control as well as standard drug Glibenclamide treated group. In this study an attempt was made to provide scientific backup to the traditional claim. Since the results exhibited a significant anti-diabetic activity in albino rats, so the traditional use of *Woodfordia fruticosa* Linn may be justified for the Antihyperglycemic activity.

Key words: Antihyperglycemic agents; Diabetes mellitus; STZ; Plant flower extracts; *Woodfordia fruticosa*.

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1. Introduction

There are over 300,000 known plants species and more are still being identified. Only 5,000 of these species have been studied for medicinal usefulness. The

World Health Organization (WHO) reports that 85% of the world's population still uses herbs as their main form of medical treatment [1].

A herb is a plant or plant part valued for its medicinal aromatic or savory qualities. Much of the medicinal use of the plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined Herbal pharmacopoeias [2].

Diabetes mellitus is one of the five leading causes of death [3] and its prevalence has increased exponentially. In fact, nearly 150 million people worldwide are diabetic, with that number expected to increase to 355 million by 2030 [4]. Most importantly, the management of diabetes without any side effects is still a challenge [5].

Of several plant species *Woodfordia fruticosa* (Linn.) is also believed to have anti-diabetic potential. Its chemical constituents are flavonoids and tannins (Leaves, twigs and immature fruits). Its bark is being used in Garhawal (India) for treatment of menorrhagia. The flowers are being used in the preparation of Ayurvedic fermented drugs called 'Aristhas' and 'Asavas', and very popular in the Indian subcontinent and other South Asian countries. A popular crude drug (called 'Sidowaya' or 'Sidawayah') of Indonesia and Malaysia chiefly contains dried flowers of *Woodfordia fruticosa*. It has been used as an astringent to treat dysentery and sprue, and also for the treatment of bowel complaint, rheumatism, dysuria, hematuria and infertility in many South East Asian countries [6].

Like other countries, the use of herbal medicine has increased enormously in INDIA over the last few decades. Although hypoglycemic studies on *Woodfordia* have been done but relatively few studies encompass full physiological effect. The current study investigated

acute and chronic exposure of crude extracts of *Woodfordia fruticosa* in experimentally induced diabetes on a more scientific and biochemical basis [7].

2. Materials and methods

Animals and maintenance

Albino male wistar rats weighing 150-200 g were obtained from Venkateshwar animal house, Bangalore, Karnataka. The animals were housed in polypropylene cages in standard environmental conditions, 12h light and dark cycles at $25 \pm 2^{\circ}\text{C}$. During the experiments the rats were fed with standard diet and water ad libitum. Animals were acclimatized to the laboratory condition for 10 days prior to the experiment and were maintained in a well ventilated animal house. The experimental protocol was approved by the Institutional animal ethical committee (IAEC) SET's College of Pharmacy, Dharwad, Karnataka (REG.NO) and care of the animals were taken as per the CPCSEA regulation.

Procurement and authentication of plant

The dried flower of *Woodfordia fruticosa* was collected from Kumta district in the month of September 2011. The collected material was authenticated by prof. Ganesh Hegde, taxonomist, Department of botany, Karnataka university, Dharwad, India.

Induction of Diabetes mellitus

Diabetic condition was induced in male wistar rats by single i.v injection of STZ (40 mg/kg) after overnight fasting for 12 hr. Rats showing SG level >200 mg/dl seven days after STZ administration were considered diabetic and included in the study [11].

Experimental design and dosage**Experimental design for single dose one day study**

The experimental rats were divided into seven groups of six animals each.

Group 1: Normal control received distill water (10 ml/kg, p.o)

Group 2: Diabetic control received water (10 ml/kg,p.o)

Group 3: Normal rats treated with Glibenclamide (10 mg/kg,p.o)

Group 4: Normal rats treated with WFE (100 mg/kg.p.o)

Group 5: Normal rats treated with WFE (200 mg/kg.p.o)

Group 6: Normal rats treated with WFA (100 mg/kg.p.o)

Group 7: Normal rats treated with WFA (200 mg/kg.p.o)

Blood samples were collected at 0, 2, 4 and 6 hrs after woodfordia fruticosa /GLB administration. Serum glucose (SG) levels were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer. Percentage reduction in glycemia was calculated with respect to the initial 0hr level by above mentioned formula.

Experimental design for Multiple-dose fifteen day study

The animals were treated with respective doses of Woodfordia fruticosa ethanolic and aqueous extracts (WFE and (WFA) and were further treated with single daily doses for another 15 days in order to evaluate the chronic effect of GLB/ Woodfordia fruticosa treatment on hyperglycemia [12].

The experimental rats were divided into seven groups of six animals each.

Group 1: Normal control received distill water (10 ml/kg, p.o)

Group 2: Diabetic control received water (10 ml/kg,p.o)

Group 3: Normal rats treated with Glibenclamide (0.5 mg/kg,p.o)

Group 4: Normal rats treated with WFE (100 mg/kg.p.o)

Group 5: Normal rats treated with WFE (200 mg/kg.p.o)

Group 6: Normal rats treated with WFA (100 mg/kg.p.o)

Group 7: Normal rats treated with WFA (200 mg/kg.p.o)

Determination of acute toxicity

The acute oral toxicity study was carried out as per the guidelines set by OECD.423. Animals were fasted overnight prior to dosing and were treated with different doses 5, 50, 300 and 2000 mg/kg b.w. The test substance was administered in a single dose using canula. Animals were observed individually after dosing atleast once during the first 30 mts, periodically during the first 24 hrs, with special attention given during first four hrs and daily upto 14 days. Animals were observed for any abnormal behavior, mortality or death [10].

Estimation of Biochemical parameters

At the end of the treatment schedule, blood samples were collected from retro-orbital plexus. Serum was separated and analysed spectrophotometrically for triglyceride (STG), total cholesterol (STC), HDL-cholesterol (HDL-C) using diagnostic reagent kit, ERBA diagnostics, Germany. Serum insulin (SI) was estimated by radioimmunoassay method using kit from Baba atomic research centre, Mumbai, India. VLDL-cholesterol (VLDL-c) and LDL-cholesterol (LDL-c) in serum were calculated as per Friedewald's equation [13] [14].

$$\text{VLDL-c} = \frac{\text{Triglyceride}}{5}$$

$$\text{LDL-c} = \frac{\text{Total cholesterol} - \text{Triglyceride}}{5} - \text{HDL-c}$$

The markers of dyslipidemia such as Tc/HDL-c and LDL-c/HDL-c ratios were also calculated.

Histopathological study

Processing of isolated pancreas

The isolated organs was cut into small pieces and preserved in formalin (10% solution) for at least two days. The pancreas was washed in running water for about 12 hrs. This was followed by dehydration with alcohol of increasing strength (70, 80 and 90%) for 12 hrs each. Again the tissue is cleaned by using Xylene 2 times for 15-20 mts each. After cleaning the organ pieces were subjected to paraffin infiltration in automatic tissue processing unit.

Embedding in paraffin

Hard paraffin was melted and was poured into square shaped blocks. The pancreas pieces were then dropped into the liquid paraffin quickly and allowed to cool.

Sectioning

The blocks were cut using microtome to get section of thickness 5 microns. The section was then taken on a microscope slide on which egg albumin (sticky substance) was applied. The sections were allowed to remain on the sticky substance for three days till it sticks firmly on the slide. The section should be dried completely before staining [16].

Staining

Eosin is an acidic stain and hematoxylin is a basic stain, which is used for staining.

Statistical analysis

All the data were expressed as mean $\pm$ SEM. Statistical analysis was carried out using one way Anova followed by Tukeys multiple comparison test [15].

3. Results

A) Effect on body weight

Throughout the experiments, body weight did not show any significant change in any of the experimental groups, treatment or otherwise.

Experimental design for single dose one day study:

B) Evaluation of anti-diabetic effect of WFE and WFA in standardized STZ induced diabetic rats

i) Single-dose one-day study

A single dose of WFE (100 and 200 mg/kg) and WFA (100 and 200 mg/kg) treatment exhibited reduction in SG levels at different time intervals compared to basal levels (0 hr). However, administration of GLB showed significant ($P < 0.05$; $P < 0.001$) reduction in SG levels with maximum reduction (50.94%) at 4 hr post GLB treatment compared to their basal levels, whereas, WFE treated animals showed dose dependent percentage reduction in SG levels compared to their basal levels (Table 1 and Figure 1)

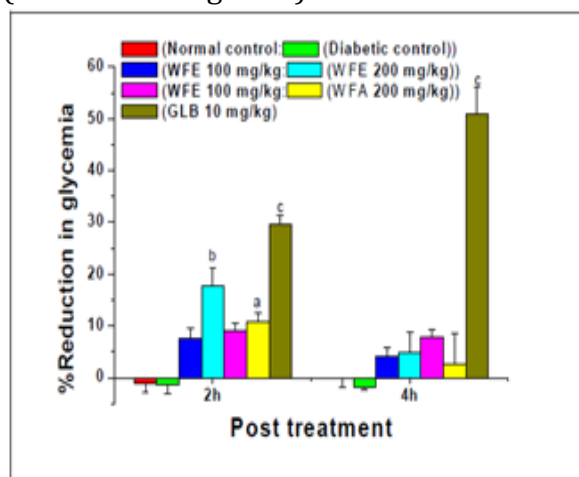


Figure 1. Effect of ethanol (WFE) and water extract (WFA) of *Woodfordia fruticosa* on SG levels in STZ-induced rats [Single-dose one-day study]. Bar graph represents the percentage reduction in glycemia with respect to the initial (0 hr) level. Each value represents Mean S.E.M., $n = 5$. $ap < 0.05$; $bp < 0.01$; $cp < 0.001$ compared to diabetic control of the same time interval. One-way ANOVA followed by Tukey's post-test.

Treatment [dose/kg b.w]	Table 1 SGL levels [mg/dl]		
	0 h	2 h	4 h
Normal control	86.38±3.15	87.18±3.31 [-1.0]	86.4±2.8 [-1.0]
Diabetic control (DC)	344.01±29.26	348.5±29.3 [-1.37]	350.65±29.2 [-1.98]
DC+WFE[100 mg]	337.5±20.4	311.2±24.2 [-7.6]	298.0±20.9 [11.3]
DC+ WFE [200 mg]	331.7±24.3	277.7±22.8 [17.8]	260.3±26.8 [22.1]
DC+ WFA [100 mg]	282.3±24.0	255.8±14.3 [8.9]	237.0±23.6 [16.1]
DC+ WFA [200 mg]	286.31±19.4	257.0±18.1 [10.8]	253.4±24.2 [13.0]
DC+GLB [10 mg]	342.3±28.3	24.07±18.25 [29.52]	160.04±8.29 [50.94]

Table1. Effect of ethanol (WFE) and water extract (WFA) of *Woodfordia fruticosa* on SGL levels in STZ-induced MD rats [Single-dose one-day study]

Treatment [dose/kg b.w]	Table 2 SGL levels [mg/dl]			
	0 day	7 day	10 day	15 day
Normal control	86.38±3.15	87.95±4.2 [-1.77]	91.45±3.7 [-6.75]	98.98±4.9 [-15.64]
Diabetic control (DC)	344.01±29.26	323.7±18.64 [5.89]	345.42±15.95 [-0.78]	388.97±12.98 [-14.76]
DC+WFE[100 mg]	337.5±20.4	265.0±23.6 [20.8]	256.7±19.4 [23.4]	250±14.9 [24.6]
DC+ WFE [200 mg]	331.7±18.5	269.9±18.8 [20.2]	237.4±11.5 [28.5]	243.3±10.5 [24.7]
DC+ WFA [100 mg]	282.3±21.5	237.2±9.6 [15.5]	211.1±14.0 [24.8]	210.3±9.5 [23.9]
DC+ WFA [200 mg]	286.31±11.3	240.73±12.1 [17.4]	235.62±10.2 [18.6]	225.6±8.4 [21.9]
DC+GLB [10.5 mg]	342.3±28.3	275.5±8.22 [19.2]	247.9±8.9 [27.57]	195.0±10.4 [43.0]

Table 2. Effect of ethanol (WFE) and water extract (WFA) of *Woodfordia fruticosa* on SG levels in STZ-induced MD rats [Multiple-dose fifteen-day study]

Serum Parameter	Normal control	Diabetic control (DC)	WFE [100 mg/kg]	WFE [200 mg/kg]	WFA [100 mg/kg]	WFA [200 mg/kg]	GLB [0.5 mg/kg]
STG (mg/dl)	84.9±3.13	131.9±7.5	84.9±3.13	84.04±5.5	62.2±13.0	88.9±4.3	91.9±6.7
STC (mg/dl)	68.41±3.9	126.62±4.7	68.41±3.13	68.38±3.8	59.9±8.9	70.5±3.2	82.9±6.9
HDL-c (mg/dl)	34.1±2.0	14.52±1.8	30.03±3.24	28.75±3.8	19.3±1.6	25.2±3.2	26.6±1.9
VLDL-c (mg/dl)	12.8±2.7	26.35±1.5	16.98±1.1	16.81±1.1	12.4±2.6	17.8±0.9	18.4±1.3
LDL-c (mg/dl)	16.3±3.3	85.75±3.4	21.4±5.46	23.12±6.5	28.1±5.8	27.6±5.6	37.9±8.5
TC/HDL-c ratio	1.9±0.1	9.17±1.2	2.35±2.57	2.57±0.5	3.1±0.4	2.9±0.5	3.2±0.4
LDL-c/HDL-c ratio	0.5±0.1	6.26±0.9	0.77±0.21	0.96±0.4	1.5±0.3	1.2±0.4	1.5±0.15

Table 3. Effect of ethanol (WFE) and water fraction (WFA) of *Woodfordia fruticosa* on Lipid Profile in STZ-induced model (Multiple Dose fifteen-days Study)

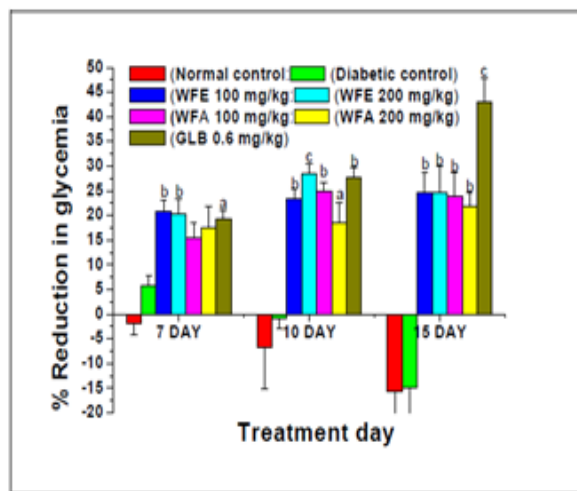


Figure 2. Effect of ethanol (WFE) and water extract (WFA) of *Woodfordia fruticosa* on SG levels in STZ-induced diabetic rats [Multiple-dose fifteen-day study]. Bar graph represents the percentage reduction in glycaemia with respect to the initial (0 day) level. Each value represents Mean S.E.M., n=5. a P<0.05; b P<0.01, c P<0.001 compared to diabetic control of the same time interval. One-way ANOVA followed by Tukey's post-test.

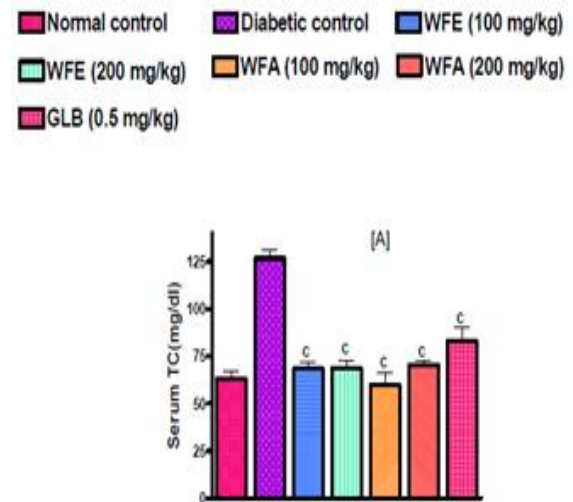


Figure 3. Effect of Fifteen-day treatment with ethanol (WFE) and water (WFA) extract of *Woodfordia fruticosa* on [A] Serum TC [B] Serum TG [C] Serum HDL-c [D] VLDL-c [E] LDL-c [F] TC/HDL-c [G] LDL-c/HDLc levels in STZ-induced diabetic rats. Each bar represent the Mean S.E.M. (n = 5). a P < 0.05; c P < 0.001 compared with diabetic control.

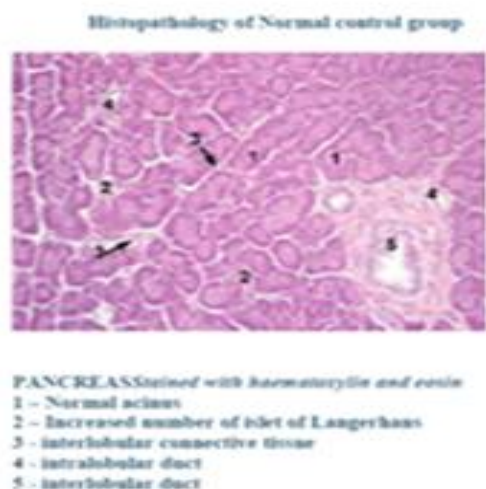


Figure 4. Histopathology of Normal control group

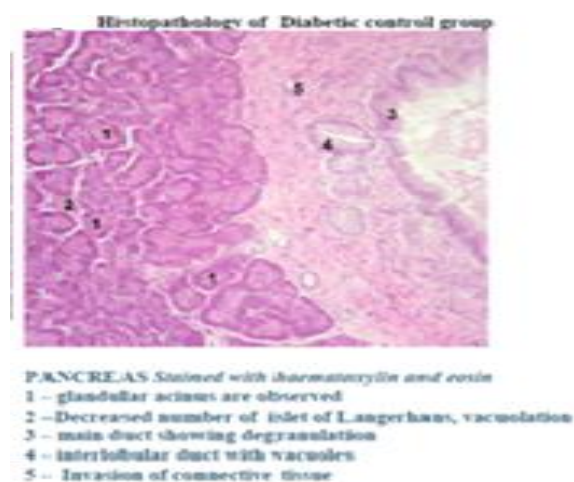


Figure 5. Histopathology of Diabetic control group

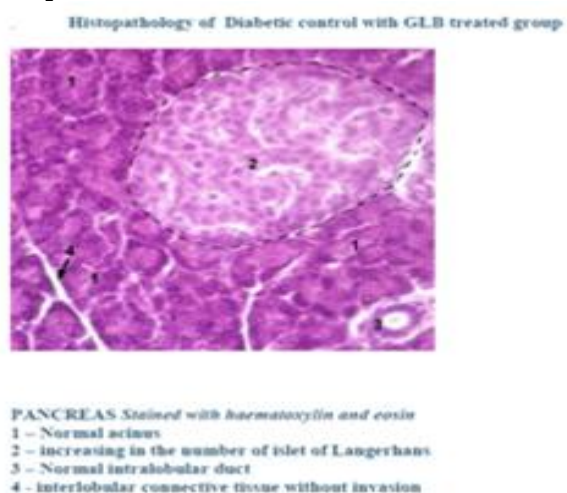


Figure 6. Histopathology of Diabetic control with GLB treated group

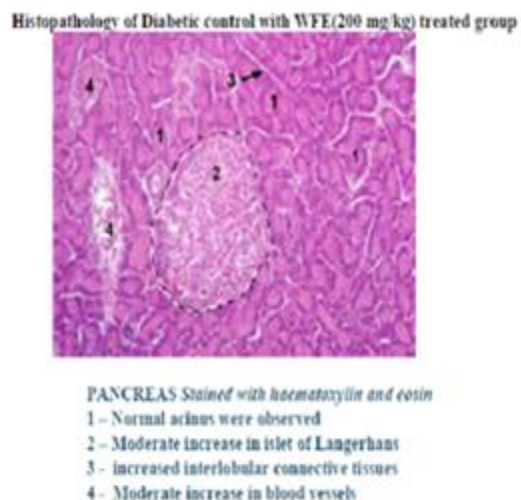


Figure 7. Histopathology of Diabetic control with WFE (200 mg/kg) treated group

Experimental design for Multiple-dose fifteen day study:

Repeated administration of WFE (100 and 200 mg/kg) and WFA (100 and 200 mg/kg) for 15 days, showed significantly ($P < 0.05$; $P < 0.01$) reduced levels of SG compared to respective basal values (0 day) (Table 1) On 15th day, tested doses of WFE and WFA showed significantly ($P < 0.001$) greater percentage reduction in glycemia (24.6%:24.7% and 23.9%:21.9% respectively) compared to diabetic control (Figure 2)

Estimation of Biochemical parameters

Diabetic rats showed significantly ($P < 0.001$) increased levels of STG, STC, VLDL-c and LDL-c levels, whereas HDL-c was decreased in diabetic rats compared to normal rats. The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were significantly elevated in the diabetic group. Oral administration of different doses of WFE and WFA for fifteen-days exhibited significant reduction ($P < 0.001$) in all tested lipid parameters and restoring them to near-normal values (Table 3 and Figure 3).

Histopathological study**Histopathological examination**

Figure 4-7 Depicts the histomorphological change in pancreas of different groups of treated animals. Histopathological examination of pancreas from normal control group revealed normal pancreatic acini and islet of langerhans with normal cellularity (Figure 4). Whereas decreased number of pancreatic islets, vacuolation, hydropic and necrotic cells, degranulation of cells and invasion of connective tissues were detected in the diabetic rats (Figure 5). *Woodfordia fruticosa* (100 and 200 mg/kg) and standard drug GLB (Glibenclamide) markedly succeeded in amending the disrupted islets of langerhans of diabetic rats. Islets architecture, integrity, number and size of pancreatic islets were Improved (Figure 6 and 7).

4. Discussion

Diabetes mellitus is a metabolic disease as old as mankind and is characterized by hyperglycemia associated with impairment in insulin secretion/action along with altered carbohydrate, protein and lipid metabolism. The function of insulin is to maintain normal blood glucose levels either by suppression of glucose output from liver or by the stimulation of glucose uptake and its metabolism. Insufficient release of insulin or loss of insulin action at target tissues causes abnormal glucose and lipid metabolism. This results in elevated glucose levels in blood, the hallmark of diabetes. Type-1 diabetes results from autoimmune destruction of pancreatic b-cells resulting in insulin deficiency. Before the introduction of insulin in 1922 the treatment of diabetes mellitus relied heavily on dietary measures which included the use of traditional plant therapies. Many traditional plant treatments for diabetes exist. However, few have received scientific or medical

scrutiny and the World Health Organization has recommended that traditional plant treatments for diabetes warrant further evaluation. Insulin therapy affords effective glycemic control, yet its drawbacks such as ineffectiveness on oral administration, short shelf life, need for constant refrigeration and hypoglycemia on excess dosage limits its usage. Therefore efforts continue to find insulin substitutes from synthetic or plant sources.

Numerous animal models have been developed to mimic human disease states. The underlying assumption in using these animal models in medical research is that they will provide additional knowledge about and insight into disease processes and, hopefully, better methods for treatment or prevention of diseases in humans. Wessler defines an animal model as "a living organism with an inherited, naturally acquired, or induced pathological process that in one or more respects closely resembles the same phenomenon occurring in man". He emphasizes that animal models offer only approximations of human disease and can never actually duplicate the same process. In attempting to approximate type 1 diabetes in animals, both spontaneous and induced models have been developed. The means by which the disease is induced or how it develops in an animal is important in determining how the model is to be studied. STZ-induced experimental diabetes is a valuable model for induction of type 1 diabetes. Further it is generally accepted that severe diabetes (SD) is similar to type 1 and mild diabetes (MD) is similar to type 2 diabetes.

To our knowledge, this is the first detailed study to investigate the effect of ethanol (WFE) and water (WFA) extracts of *Woodfordia fruticosa* on the glucose levels, lipid profile in STZ-induced diabetic rats. The severity and insulin resistance

(reduced peripheral utilization of glucose) condition in diabetic rats induced by STZ (50 mg/kg, i.v) was confirmed by lower insulin levels post glucose-challenge, HOMA (measure of insulin resistance) values and lack of glucose uptake by isolated hemidiaphragm of diabetic rats.

Both WFE and WFA produced hypoglycemia and improved glucose tolerance in normal rats in spite of counter regulatory factors avoiding reduction in blood glucose levels. Therefore hypoglycemic activity of WFE and WFA could be mediated by stimulation of surviving β -cells to release more insulin and may be through extra pancreatic mechanisms. STZ is well known for its selective pancreatic islet β -cell cytotoxicity and has been extensively used to induce DM in animals. It interferes with cellular metabolic oxidative mechanisms. The present data suggested that WFE and WFA significantly reduced hyperglycemia in both single-dose one-day and multiple dose fifteen-day diabetic studies. The efficacy of the WFE is better than WFA. This could be mediated by improving the glycemic control mechanisms (extra-pancreatic) and increasing insulin secretion from remnant pancreatic β -cells in diabetic rats.

DM is often linked with abnormal lipid metabolism. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. It has been demonstrated that insulin deficiency in diabetes leads to a variety of disruptive changes in metabolic and regulatory processes, which in turn lead to accumulation of lipids. It has also been shown that insulin significantly normalizes lipid levels in diabetic rats. The extract supplementation also results in significant attenuation in STG, STC, VLDL-c, and LDL-c. These effects may be due to low activity of cholesterol biosynthesis enzymes or low levels of

lipolysis. Increased TC/HDL-c and LDL-c/HDL-c ratios are well known markers of dyslipidemia in STZ-induced diabetic rats. WFE and WFA administration reinstated dyslipidemic markers to near-normal values.

The phytochemical examination of ethanol (WFE) and water (WFA) extract of *Woodfordia fruticosa* revealed the presence of flavanoids, tannins, steroids and glycosides as major phytoconstituents. Several authors have reported flavanoids, phenolics and steroidal glycoside as bioactive antidiabetic principles. The observed antihyperglycemic and hypolipidaemic activity of title plant may be attributed to the presence of these bioactive principles and their synergistic properties. Therefore we conclude that the ethanol (WFE) and water (WFA) extract of *Woodfordia fruticosa* flower has endowed with anti-diabetic (single-dose one-day study and multi-dose fifteen-day study), anti-hyperlipidaemic activity in standardized STZ-induced diabetic rats, justifying its use in the traditional system of medicine.

Conclusion

Preliminary phytochemical investigation of *Woodfordia fruticosa* Linn, alcoholic and aqueous extracts (WFA and WFE) revealed the presence of Glycosides, steroids, tannins, flavonoids, proteins and aminoacids as major phytoconstituents.

The WFA and WFE (100 and 200 mg/kg) is beneficial in controlling diabetes by controlling glucose, increasing the level of insulin and combating oxidative stress by activation of hepatic antioxidant enzymes. Normalization of lipid profile may due to increase in secretion of insulin from pancreatic β -cells by the plant extracts. The present study justifies the traditional claim that flowers of *Woodfordia fruticosa* are used for the treatment of diabetes. The results of the investigations justify that

Woodfordia fruticosa flower part of alcoholic and aqueous extracts can be used in the treatment of anti-diabetic activity in streptozotocin induced diabetic rats. Further the plant is worth for chemical and pharmacological investigations.

Acknowledgment

The authors are thankful to the Postgraduate Department of Pharmacognosy, SETCP, Dharwad for guiding and positive feedback during the course of the work. Authors are also thankful to Head of the Department for providing necessary facilities at Department of Botany and Central lab.

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