Antihyperglycemic activity of *Woodfordia fruticosa* (Kurz) flowers extracts in glucose metabolism and lipid peroxidation in streptozotocin-induced diabetic rats

Neeraj Verma1*, G Amresh1, P K Sahu2, Ch V Rao3 & Anil Pratap Singh3

1Department of Pharmacology, Goel Institute of Pharmacy & Sciences, Faizabad Road (Near Indira Canal), Lucknow 227 105, India
2Department of Pharmacology, School of Pharmaceutical Sciences, Siksha ‘O’ Anusandhan University, Kalinga Nagar, Ghatikia, Bhubaneswar 751 003, India
3Pharmacognosy and Ethnopharmacology Division, CSIR-National Botanical Research Institute, Rana Pratap Marg, P.O. Box No. 436, Lucknow 226 001, India

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The ethanolic extract of *W. fruticosa* flowers (250 and 500 mg/kg) significantly reduced fasting blood glucose level and increased insulin level after 21 days treatment in streptozotocin diabetic rats. The extract also increased catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase activities significantly and reduced lipid peroxidation. Glycolytic enzymes showed a significant increase in their levels while a significant decrease was observed in the levels of the gluconeogenic enzymes in ethanolic extract treated diabetic rats. The extract has a favourable effect on the histopathological changes of the pancreatic β-cells in streptozotocin induced diabetic rats. The results suggest that *W. fruticosa* possess potential antihyperglycemic effect by regulating glucose homeostasis and antioxidant efficacy in streptozotocin-induced diabetic rats.

Keywords: Antihyperglycemic, Antioxidant, Diabetes mellitus, Streptozotocin, *Woodfordia fruticosa*

*Woodfordia fruticosa* Kurz (Family: Lythraceae) locally known as Dhai is about 3.5 m high occurring throughout North India1. The flowers are brilliant red in colour and reported for immunomodulatory2, antitumor activity3, hepatoprotective activity4, antiulcer activity5 and also useful in diarrhoea6, urinary disorders, burning sensation, wounds, bleeding injuries and headache7. Tewari et al8 have demonstrated the anti-leucorrhoeic property of an ayurvedic preparation containing *W. fruticosa*. Chemically, oenothein B and woodfordin A, B and C9, Isochimacoalin-A and five oligomers—woodfordin E, F, G, H, I were reported from dried flowers9. Quercetin-3-O-(6″-galloyl)-β-D-galactopyranoside, quercetin-3-O-α-L-arabinoside, quercetin-3-O-oxypyanoside, myricetin-3-O-(6″-O-galloyl)-β-D-galactopyranoside and myricetin-3-O-arabinopyranoside were isolated from dried flowers and their structure elucidated5. A process patent for preparing

Asava and Aristha composition was taken by Brindavanam et al10, which demonstrated its usefulness for diabetics and calorie conscious non-diabetics. Based on its diversified ethnopharmacological/folklore uses and use of *W. fruticosa* (WF) in diabetes by rural Nepalese people11, present study has been undertaken to validate its antihyperglycemic activity in streptozotocin induced diabetic rats.

**Materials and Methods**

*Plant material*—Fresh flowers of *W. fruticosa* were obtained as gift from M/s Aroma Chemicals, Saharanpur, UP, India. The flowers were identified and authenticated at source by Dr. A.K.S. Rawat (Pharmacognosy and Ethnopharmacology Division, CSIR-National Botanical Research Institute, Lucknow). A voucher specimen (No. NBRI/CIF/174/2010) has been deposited in the herbarium of the institute for future reference.

*Chemicals*—All chemicals used in the study were of analytical grade and obtained from Sigma Chemicals Co., USA and Merck India Ltd., Mumbai.

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*Correspondent author*
Telephone: +91- 522 6568698
Fax: +91-522 4077041
E-mail: neerajcology@gmail.com
Rat insulin ELISA kit was purchased from Crystal Chem Inc. (Downers Grove, US).

Preparation of extracts—The flowers were shade dried and powered by mechanical grinder. The powdered flowers (500 g) were extracted at ambient temperature (22-25 °C) successively with petroleum ether, chloroform, ethanol (95%) and finally with water by solvent-solvent extraction method. The petroleum ether, chloroform and ethanol from the pooled extracts were removed using Buchi Rotavapour under reduced pressure at 40-55 °C to afford petroleum ether extract (PE, 3.1 g), chloroform extract (CE, 4.9 g), and ethanolic extract (EE, 102.5 g) respectively, whereas aqueous extract (AE, 160.6 g) of WF was obtained by lyophilizer. The extracts were kept in freeze dryer for further study.

Experimental animals—Male Wistar albino rats (180-220 g) purchased from the animal house of CDRI, Lucknow were kept in departmental animal house in an environmentally controlled room (25±3 °C), relative humidity 50±5% RH and on a 12:12 h L:D cycle at NBRI, Lucknow. They were allowed free access to standard rat feed (Dayal, India) and water ad libitum. Experiments were performed after one week of acclimatization. All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No.222/2000/CPCSEA).

Preliminary study for identification of effective extract based on oral glucose tolerance test (OGTT) in non-diabetic rats—The oral glucose tolerance test (OGTT) was performed in overnight fasted (18 h) normal rats. The hepatoprotectant property of W. fruticosa flowers was reported by Chandan et al. at a dose of 250 mg/kg body weight po. Therefore, 250 mg/kg body weight of WF flowers extract was administered orally. Rats were divided into following 6 groups of six rats each. Gr. I, normal control (0.2% gum acacia in distilled water); Gr. II, diabetic control rats treated with 0.2% gum acacia in distilled water; Gr. III (DE1): diabetic rats treated with 250 mg/kg body weight of EEWF flowers; Gr. IV (DE2): diabetic rats treated with 500 mg/kg body weight of EEWF flowers; and Gr. V (DG): diabetic rats treated with 20 mg /kg body weight of glybenclamide. The ethanolic extract and glybenclamide were administered to the animals of the respective groups every day morning for 21 days by using orogastric cannula. After an overnight fasting the animals in all the 5 groups were sacrificed on the day 22 by cervical dislocation and then blood samples were collected. Blood glucose level was measured by glucose oxidase-peroxidase reactive strips and plasma insulin level was measured by using rat insulin ELISA kit. Body weights of all the animals were recorded prior and after the treatment.

Liver antioxidant status and lipid peroxidation level—Liver was removed, washed with ice cold normal saline and stored at −80 °C. Homogenate (10% w/v) was prepared in ice cold phosphate buffer saline, pH 7.5 and centrifuged at 10,000 rpm at 4 °C for 20 min. The resulting supernatants were then used for biochemical measurements. Activities of catalase (CAT; EC: 1.11.1.6), glutathione reductase (GR; EC: 1.8.1.7) and glutathione peroxidase
(GPx; EC: 1.11.1.9) were determined. The activity of superoxide dismutase (SOD; EC: 1.15.1.1) was assayed by the method of Kakkar et al. The pink coloured malondialdehyde-thiobarbituric acid (MDA-TBA) adducts was read at 532 nm and all nonspecific adducts at 600 nm. Lipid peroxidation was expressed as μmol of MDA conjugate formed/g of liver. Glutathione (GSH) was determined by the method of Ellman and expressed as nmol DTNB conjugate/mg liver.

Carbohydrate metabolism enzyme assays and glycogen content in liver—Activities of liver glucose-6-phosphate dehydrogenase (G6D; EC: 1.1.1.49), glucose-6-phosphatase (G6P; EC: 3.1.3.9) and hexokinase (HK; EC: 2.7.1.1) were assayed. For estimation of glycogen, 10% liver homogenate in 80% hot ethanol was centrifuged at 8000 rpm for 20 min. The residue was collected and allowed to dry over a water bath. The residue, 5 ml of distilled water and 6 ml of 52% perchloric acid were added. Extraction was done at 0 °C for 20 min, centrifuged at 8000 rpm for 15 min and supernatant was collected. To 1 ml of 5-fold diluted supernatant, 4 ml of freshly prepared anthrone reagent was added and heated on a boiling water bath for 20 min. Absorbance was recorded at 630 nm, a standard curve was also plotted with glucose solution.

Histological sample preparation—The whole pancreas was removed after sacrificing each animal and was collected in 10% neutral buffered formalin (NBF) solution. The samples were processed in graded series of alcohol and embedded in paraffin wax. Sections of 5 μm thickness were cut and stained by hematoxylin and eosin (H&E) for histological examination.

Statistical analysis—The data were presented as mean±SE and analysed by using one-way ANOVA followed by Duncan’s multiple range test. A value of P < 0.01 was considered to be statistically significant.

Results

Effect of W. fruticosa flowers extracts on oral glucose tolerance test (OGTT) in non-diabetic rats—Peak blood glucose level reached at 30 min after glucose administration and reduction in blood glucose was observed at 120 min in extracts and glybenclamide treated rats. Among the four extracts, EE (250 mg/kg body weight) significantly reduced blood glucose by 53.21% and glybenclamide by 64.08% in 120 min (Fig. 1). Therefore, it was considered as effective extract.

Evaluation of antihyperglycemic activity of EE—The blood glucose levels were significantly (P < 0.01) increased in diabetic rats as compare to normal rats. Treatment with ethanolic extract (EE) at the doses of 250 and 500 mg/kg body weight showed significant (P < 0.001− < 0.0001) reduction (40.39 and 69.31% respectively) in fasting blood glucose level after 21 days treatment in streptozotocin induced diabetic rats. Standard drug glybenclamide also showed significant (P<0.0001) reduction (72.50%) in fasting blood glucose level. Plasma insulin was significantly (P<0.01) decreased in diabetic rats and significantly (P<0.001− < 0.0001) improved in diabetic rats treated with EE at both the doses and with standard drug glybenclamide (Fig. 2).

Effect of EE on body weight—Body weight of vehicle treated diabetic rats significantly decreased during study period as compared to normal rats. Ethanolic extract (250 and 500 mg/kg body weight) significantly prevented decrease in body weight of diabetic rats. Standard drug glybenclamide (20 mg/kg body weight) also improved body weight significantly.

Effect of EE on liver antioxidant status and lipid peroxidation levels—in diabetic liver, the activities of SOD and CAT were decreased by 58.96 and 46.50% as compared to normal rats; EE increased
SOD activity by 45.45% at dose level of 250 mg/kg and 88.51% at a dose of 500 mg/kg body weight. The catalase activity was recovered 42.70 and 67.53% with the treatment of EE at a dose of 250 and 500 mg/kg body weight respectively. Glutathione peroxidase and glutathione reductase activity were diminished by 51.44 and 57.12% respectively which were significantly increased in diabetic rats receiving EE with the dose of 250 and 500 mg/kg body weight. The reduced glutathione content was also lowered by 57.26% in diabetic rats which was increased significantly by 72.61% in animals treated with EE at a dose of 500 mg/kg body weight. Lipid in terms of TBARS/MDA was increased significantly in diabetic rats as compared to normal control animals. The maximum dose (500 mg/kg body weight) of EE in diabetic rats decreased MDA level by 51.94%. These effects in EE treated rats were nearly similar to standard drug glybenclamide (20 mg/kg body weight) treated rats (Table 1).

**Table 1**—Effect of 250 and 500 mg/kg body weight of EE of *W. fruticosa* flowers on hepatic antioxidant enzymes and lipid peroxidation levels after 21 days treatment. [Values are mean ± SE from 6 observation each]

<table>
<thead>
<tr>
<th>Groups</th>
<th>CAT</th>
<th>SOD</th>
<th>GR</th>
<th>GPx</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>64.6±0.6</td>
<td>212.3±11.2</td>
<td>14.3±0.3</td>
<td>4.9±0.4</td>
<td>26.23±0.1</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td>DC</td>
<td>34.5±1.8a</td>
<td>87.1±6.7a</td>
<td>7.8±0.6a</td>
<td>2.4±0.2a</td>
<td>11.21±0.2a</td>
<td>0.77±0.03a</td>
</tr>
<tr>
<td>(-46.50)</td>
<td>(-58.96)</td>
<td>(-44.81)</td>
<td>(-51.07)</td>
<td>(-57.26)</td>
<td>(+63.63)</td>
<td></td>
</tr>
<tr>
<td>DE1</td>
<td>49.3±2.1b</td>
<td>126.6±8.9b</td>
<td>10.9±0.9b</td>
<td>2.9±0.6b</td>
<td>16.13±0.2b</td>
<td>0.43±0.11b</td>
</tr>
<tr>
<td>(+42.71)</td>
<td>(+45.45)</td>
<td>(+38.14)</td>
<td>(+18.48)</td>
<td>(+43.88)</td>
<td>(-44.15)</td>
<td></td>
</tr>
<tr>
<td>DE2</td>
<td>57.9±2.6c</td>
<td>164.2±5.7c</td>
<td>12.4±0.7c</td>
<td>3.6±0.3c</td>
<td>19.35±0.1c</td>
<td>0.37±0.03c</td>
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<tr>
<td>(+67.53)</td>
<td>(+88.51)</td>
<td>(+57.12)</td>
<td>(+51.44)</td>
<td>(+72.61)</td>
<td>(-51.94)</td>
<td></td>
</tr>
<tr>
<td>DG</td>
<td>58.2±1.1c</td>
<td>168.5±9.7c</td>
<td>12.9±0.3c</td>
<td>3.8±0.3c</td>
<td>20.67±0.2c</td>
<td>0.32±0.07c</td>
</tr>
<tr>
<td>(+68.43)</td>
<td>(+93.49)</td>
<td>(+63.49)</td>
<td>(+60.08)</td>
<td>(+84.38)</td>
<td>(-58.44)</td>
<td></td>
</tr>
</tbody>
</table>

NC=normal control; DC = diabetic control; DE1= diabetic rats treated with 250 mg/kg EE; DE2= diabetic rats treated with 500 mg/kg EE; DG=diabetic rats treated with 20 mg/kg of glybenclamide

CAT: catalase, µM H₂O₂ decomposed/min/g liver
SOD: superoxide dismutase activity, Units/min/g liver
GR: glutathione reductase, NADPH oxidized/min/g liver
GPx: glutathione peroxidase, µM NADPH oxidized/min/g liver
GSH: reduced glutathione, nM DTNB conjugated/mg liver
MDA: lipid peroxidation, nM MDA/g liver

*P values a < 0.01 as compared to NC; b < 0.001; c < 0.0001 as compared to DC*
glybenclamide treated diabetic group showed negligible islet necrosis and fibrosis, and were confined to the periphery. The central part of the islet; necrosis and fibrosis, whereas that of diabetic rats showed diminished with paucity of cells and glycogen content in diabetic rats was decreased by 49.78% which was restored to nearly control levels in EE supplemented diabetic rats (Table 2).

**Table 2—Effect of 250 and 500 mg/kg body weight of EE of* W. fruticosa* flowers on enzymes involved in glucose metabolism and glycogen content after 21 days treatment.**

[Values are mean ± SE from 6 observation each]

<table>
<thead>
<tr>
<th>Groups</th>
<th>G6D</th>
<th>HK</th>
<th>G6P</th>
<th>Gly</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>3.65±0.19</td>
<td>3.26±0.37</td>
<td>1.35±0.11</td>
<td>6.87±0.19</td>
</tr>
<tr>
<td>DC</td>
<td>1.92±0.31a</td>
<td>1.73±0.32a</td>
<td>2.81±0.23a</td>
<td>3.45±0.14a</td>
</tr>
<tr>
<td>(−47.39)</td>
<td>(−46.93)</td>
<td>(+ 51.95)</td>
<td>(−49.78)</td>
<td></td>
</tr>
<tr>
<td>DE1</td>
<td>2.03±0.26b</td>
<td>2.08±0.28b</td>
<td>1.67±0.21b</td>
<td>4.34±0.12b</td>
</tr>
<tr>
<td>(+ 5.72)</td>
<td>(+20.23)</td>
<td>(−40.56)</td>
<td>(+ 25.79)</td>
<td></td>
</tr>
<tr>
<td>DE2</td>
<td>2.76±0.25c</td>
<td>2.37±0.09c</td>
<td>1.47±0.12c</td>
<td>6.26±0.18c</td>
</tr>
<tr>
<td>(+ 43.75)</td>
<td>(+ 36.99)</td>
<td>(− 47.68)</td>
<td>(+ 81.44)</td>
<td></td>
</tr>
<tr>
<td>DG</td>
<td>2.94±0.29c</td>
<td>2.87±0.21c</td>
<td>1.49±0.08c</td>
<td>6.29±0.16c</td>
</tr>
<tr>
<td>(+ 53.12)</td>
<td>(+ 65.89)</td>
<td>(− 46.97)</td>
<td>(+ 82.31)</td>
<td></td>
</tr>
</tbody>
</table>

NC=normal control; DC=diabetic control; DE1=diabetic rats treated with 250 mg/kg EE; DE2= diabetic rats treated with 500 mg/kg EE; DG= diabetic rats treated with 20 mg/kg of glybenclamide.

G6D: glucose-6-phosphate dehydrogenase, Units/g liver
HK: hexokinase, µM NADPH decomposed/min/g
G6P: glucose-6-phosphate Pi liberated mg/min/g
Gly: glycogen (µg/g)

P values a < 0.01 as compared to NC; b < 0.001; c < 0.0001 as compared to DC.

glycogen content in diabetic rats was decreased by 49.78% which was restored to nearly control levels in EE supplemented diabetic rats (Table 2).

Effect of EE on pancreas histology—The structure of the pancreas of the normal control and diabetic rats are shown in Fig. 3 A-E. Pancreas of normal control rats showed normal islets with no fibrosis and necrosis, whereas that of diabetic rats showed diminished with paucity of cells and necrosis. EE treated diabetic group at a dose of 250 mg/kg body weight showed well-vascularised, with viable cells; viable cells were observable in the central part of the islet; necrosis and fibrosis were confined to the periphery. EE treated diabetic group at a dose of 500 mg/kg body weight showed negligible islet necrosis and fibrosis, and glycogen content in diabetic rats was decreased by 49.78% which was restored to nearly control levels in EE supplemented diabetic rats (Table 2).

Discussion

Hyperglycemia, the primary bioclinical manifestation in diabetes, is associated with the development of certain complications of diabetes24. The mechanism underlying hyperglycemia in diabetes mellitus involves increased production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues25. As STZ destroys the β cells, insulin production is decreased, and therefore, there is an increase in the blood glucose level. In the present study the oral glucose tolerance test revealed that ethanolic extract has the capacity to lower blood glucose levels.

Impairment of hexokinase activity suggests that the impaired oxidation of glucose via glycolysis, leads to its accumulation, resulting in hyperglycemia. A partial or total deficiency of insulin causes a derangement in carbohydrate metabolism that decreases the activity of several key enzymes, including glucokinase, phosphofructokinase, and pyruvate kinase26, resulting in impaired peripheral glucose utilization and augmented hepatic glucose production. Administration of EE to diabetic rats enhanced the hepatic hexokinase activity, resulting the increase in glycolysis and glucose utilization for energy production. Gluconeogenic enzyme activation is due to the state of insulin impairment because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes27. The level of plasma insulin was found to increase significantly in diabetic rats treated with EE; the increased insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1, 6-bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxykinase28. Glucose-6-phosphatase catalyses the final step of glucon-eogenesis which involves the release of glucose from glucose-6-phosphate. An increase was observed in the diabetic controls compared to normal rats. This shows decreased glucose synthesis may have resulted from increased glucose uptake as demonstrated by the increase in pyruvate kinase activity. Increase in liver glucose uptake may be due to alteration in hepatic membrane cholesterol to phospholipid ratio leading to the partial restoration of glycolysis towards normal with subsequent decrease in gluconeogenesis. The reduction in the activities of gluconeogenic enzymes can result in the decreased concentration of glucose in blood. In general, increased hepatic glucose production, plus decreased hepatic glycogen synthesis and glycolysis, are the major symptoms of type 2 diabetes that result in hyperglycemia29.

Glycogen synthesis in the rat liver and skeletal muscles is impaired in diabetes30. Also, hepatic glycogen reserves is important for whole body glucose homeostasis and is markedly low in the diabetic state31,32. The decrease in hepatic glycogen may be observed due to insufficient insulin and inactivation of glycogen synthetase system in
diabetic state\textsuperscript{33}. However, after the treatment with EE, there was a significant increase in liver glycogen levels in diabetic rats.

The change in body weight shows that EE has a significant effect in controlling the loss of body weight, which is caused during diabetes. Streptozotocin is toxic to pancreatic $\beta$-cells and is widely used for induction of experimental diabetes mellitus in animals, resulting in the production of reactive oxygen species (ROS)\textsuperscript{34}. Oxidative stress in diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of free radicals. Increased oxidative stress is believed to play an important role in the etiology and pathogenesis of chronic complications of diabetes\textsuperscript{35}.

In diabetes mellitus, high glucose can inactivate antioxidant enzymes SOD, catalase, GPx, etc. by glycating these proteins and hyperglycemia induces oxidative stress which in turn causes lipid peroxidation\textsuperscript{36-38}. Decreased CAT, SOD, GPx and GR activities and increased lipid peroxidation in diabetic animals were restored as an effect of EE supplementation, indicating the efficacy of plant extract in attenuating antioxidant status in diabetic liver. Plant extract restored the reduced glutathione content in diabetic liver which can play an important role in prevention of diabetic complications. Besides other constituents, a number of phenolic compounds have been reported from the flowers\textsuperscript{9,39}. Among the various natural antioxidants, phenolic compounds are
reported to be active, quenching oxygen-derived free radicals by donating hydrogen atom or an electron to the free radical. In the present study, the ethanolic extract showed the antihyperglycemic and antioxidant active components which act by inhibition of free radical species formation, lipid peroxidation, by reducing the activity of gluconeogenic enzymes and by increasing the glycolysis. Histopathological studies of pancreas in diabetic rats treated with extract substantiate the cytoprotective action of extract.

Based on the major outcome of the present study, it can be concluded that antihyperglycemic effect of *W. fruticosa* may be mediated through modulation of cellular antioxidant defence system and reducing the activity of gluconeogenic enzymes and by increasing the glycolysis. Thus, the present results support the traditional use of *W. fruticosa* as an antidiabetic plant.

Acknowledgement

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References


