ANTIOXIDANT ACTIVITY OF WEDELIA CHINENSIS IN ALLOXAN INDUCED DIABETIC RATS

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Summary

The present study was designed to evaluate the oxidative stress related parameters in alloxan induced diabetic animals. After 30 days treatment with Wedelia chinensis Ethanol extract (WcEe) in the diabetic experimental animals, the blood glucose, total protein and total cholesterol were analyzed. The results shown, the Wedelia chinensis Ethanol extract have firm antihyperglycaemic activity and other parameters seem to be closer to the normal. Also oxidative damage has been studied by estimating serum and liver LPO levels and these were increased and SOD, CAT and GPx activities were reduced in diabetic rats and after treatment with Wedelia chinensis Ethanol extract the LPO levels were ameliorated and antioxidant enzyme activities were enhanced. Non enzymic antioxidants such as reduced glutathione (GSH), and nitric oxide (NO) were diminished in diabetic animals and WcEe treated animals revert to normal. These results suggest that the plant Wedelia chinensis Ethanol extract has antihyperglycaemic activity and prevent oxidative stress associated with diabetes mellitus in experimental animals.

Keywords: Wedelia chinensis, Antioxidant, Allaxon, Diabetes, Superoxide dismutase, Catalase.

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Introduction

Diabetes mellitus is a metabolic disorder in the endocrine system, currently fourth leading cause of death in mankind along with cancer, cardiovascular complication and cerebrovascular disease. It is found in all parts of the world and is rapidly increasing in most parts of the world. (1). In the year 2025, India is predicted to have the larger number of people with diabetes mellitus in the world. Many pharmaceutical companies consider India as the diabetes capital in the world (2).

Oxidative stress or excessive production of reactive oxygen species is being implicated in many diseases such as cancer, atherosclerosis, ageing, diabetes etc. (3). Oxidative stress in diabetes coexists with a reduction in the anti oxidant status (4), and increase in ROS production (5) and which can increase the deleterious effects of free radicals. Increase oxidative stress has been implicated in the etiology of type–1 diabetes mellitus and of spontaneous or chemically induced diabetes mellitus in experimental animals as well as in the development of complications in all types of diabetes mellitus. Oxygen free radicals activity can initiate the peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alterations in the structure and functions of collagen, basement and other membranes, and play a role in the long-term complications of diabetes mellitus (6).

The management of diabetes mellitus is considering a global problem and successful treatment is yet to be discovered. The modern drugs including insulin and oral hypoglycaemic agents control the blood sugar level as long as their regularly administered and they also produce a number of undesirable effects. Drug with multiple mechanism of protective action including antioxidant properties may be one way formed in minimizing tissue injury in human disease (7). External supplementation through antioxidants is recommended to protect cells of pancreas from the deleterious effects of such oxidative stress condition in diabetes mellitus (8). In general, plants and plant products are exhibits medicinal properties. Several Indian plants are also being used in Ayurvedic and Siddha medicines. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparation has been found to be effective in the treatment of diseases (9). The ethano botanical information reports about eight hundred medicinal plants that may posses antidiabetic potential (10) and some forecasters reported that the many number of medicinal plant extracts for their potential as antioxidants (11).

Plants of the genus Wedelia afford complex constituents, many of which have been used in traditional folk medicine throughout the world. Phytochemical investigations of various Wedelia species have revealed that many components from this genus express significant biological and pharmacological activities (12). *Wedelia chinensis* furnished the two known flavonoid glycosides, hydrazine derivatives (13), new isoflavone,
new compound (9E,11Z,13E)-8,15- dioxooxadeca-9,11,13-trienoic acid, caffeic acid derivatives, etc., (14). The aqueous extract from *Wedelia chinensis* exhibits strong antipyretic-analgesic activity (15). No detailed study has been carried out on the efficacy of *Wedelia chinensis* in the modulation of oxidative stress associated with diabetes mellitus in experimental animals. Hence, the present study was undertaken to investigate possible hypoglycaemic/antihyperglycaemic and antioxidant effects of ethanolic extract of *Wedelia chinensis* in alloxan induced diabetic experimental animals.

**Materials and Methods**

**Collection of Plant materials**
Leaves of *Wedelia chinensis* (Osbeck) Merr, (Compositae, tribe Heliantheae, subtribe Ecliptinae) was procured from the Rohini Herbal Garden, Sengipatt, Thanjavur, Tamil Nadu State, India. The specimen sample was dried under room temperature for 15 days and powdered.

**Preparation of Alcoholic extraction**
The plant leaves were pulverized and extracted as a whole preparation in a Soxhlet apparatus (30g/300 ml 80% aqueous ethanol). The ethanol extract was concentrated to a dry mass, lyophilized, and stored frozen at 0°C. Ethanol extract dry mass was dissolved with phosphate buffer saline and orally administered to the diabetic experimental animals.

**Collection of Blood and preparation of Tissue samples**
At the end of the experimental periods, experimental rats were sacrificed. Serum was separated by centrifuging the samples at 1500 rpm for 10 minutes and stored in a refrigerator until analyzed. The serum was analyzed for glucose, total protein, and total cholesterol. Liver is homogenized with homogenization buffer using tissue homogenizer and liver antioxidant enzymes such as superoxide dismutase, catalase, GPx, and non enzyme antioxidants were analyzed.

**Chemicals**
Alloxan and bovine serum albumin were purchased from Sigma chemical company, St. Louis, USA. All other chemicals used for biochemical analysis are purchased from Ranboxy Research Laboratories, Glaxo Laboratories, Nice Pharmaceuticals Company and Dr. Reddy’s Laboratory- India.

**Animals**
Experimental animals were healthy male Swiss Albino Rats (6-8 weeks old) having weight around (180 g – 230 g) were used for the study. They were maintained in an appropriate laboratory condition. All animals were fed standard pellet diet (Gold Mohor Rat Feed, Hindustan (p) Ltd., Mumbai) and water *ad libitum*.
**Induction of the diabetes mellitus**
The experimental animals were injected intraperitoneally with alloxan monohydrate (150mg/kg bodyweight) dissolved in normal saline (16). After 5 days, the animals were determined the blood glucose and the results were 240 – 280 mg/100 ml of blood. After induction of diabetes mellitus the animals were used for the experimental study.

**Experimental protocol**
In the present study, four groups of rats were used. Each group consists of six rats of same weight. The animals were treated for 30 days as follows.

- **Group I** – Normal Control Animals (2.0 ml normal saline only)
- **Group II** – Diabetic Animals (Alloxan monohydrate 150mg/kg bodyweight)
- **Group III** – Diabetic Treatment Control (Phosphate Buffered Saline - 2.0 ml)
- **Group IV** – Diabetic Treatment with *Wedelia chinensis* (2 mg of WcEe/kg of body weight)

**Estimation of blood glucose**
Blood glucose was estimated by o-toluidine method (17).

**Determination of cholesterol**
Cholesterol in serum was determined by the Zak method (18).

**Assay of serum and liver LPO**
Lipid peroxidation (LPO) was measured by the method of Yagi *et al.*, (1979) (19) and was expressed as nmol of MDA conjugate formed. LPO assay was done in both serum and tissue, tissue LPO was carried out by the modified method of Viana and coworkers (20).

**Assay of antioxidant enzymes and protein**
The activities of superoxide dismutase, catalase and GPx in liver homogenate were assayed by the methods of Kakkar *et al.*, 1984 (21), Maehlay and Chance, 1954 (22) and Lawrence and Burk, 1976 (23). Protein content of serum was measured by the method of Lowry *et al.*, 1951 (24).

**Assay of Non enzymic antioxidants**
The reduced glutathione and nitric oxide were analysed from liver homogenate by the method of Kao *et al.*, 1983 (25) and Green *et al.*, 1982 (26) respectively.

**Statistical analysis**
The values of the biochemical parameters were used to calculate as mean ± S.E.M. by one-way ANOVA followed by by Tukey’s Kramer multiple comparison test using GraphPad Instat software.
Results

Data on various biochemical parameters measured in the serum of control and *Wedelia chinensis* treated diabetic animals in table–1. There was the diabetic animals showed a significant increase in blood sugar level is 388.6 ± 14.8 (P < 0.001) after a few days of diabetes induction. After thirty days *WcEe* treatment, showed significantly decreased 99.1 ± 6.2 (P< 0.001) in blood glucose of the experimental animals.

Table-1: Effect of WcEe on blood parameters in experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose mg/100ml</th>
<th>Total protein mg/100ml</th>
<th>Total cholesterol mg/100ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Control Animals</td>
<td>96.1 ± 6.2</td>
<td>7.4 ± 0.4</td>
<td>86.3 ± 4.5</td>
</tr>
<tr>
<td>Diabetic Animals</td>
<td>388.6 ± 14.8**</td>
<td>5.2 ± 0.3*</td>
<td>179.6 ± 7.4**</td>
</tr>
<tr>
<td>Diabetic Treatment Control Animals</td>
<td>386.2 ± 12.3</td>
<td>5.0 ± 0.5</td>
<td>178.2 ± 6.3</td>
</tr>
<tr>
<td>Diabetic Animals Treated with <em>Wed. chinensis</em></td>
<td>99.1 ± 6.2##</td>
<td>7.3 ±0.4##</td>
<td>88.6 ± 6.5##</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey’s Kramer multiple comparison test using GraphPad Instat software, *P<0.05, **P<0.001, diabetic animals compared with control animals, #P<0.01, ##P<0.001 diabetic treated animals compared with diabetic animals.

There was a marked decrease in the plasma total protein content of untreated diabetic group 5.2 ± 0.3 (P < 0.05) when compared with that of control group animals. After thirty days treatment with *WcEe*, it shows that significantly increased and closed to normal 7.3 ± 0.4 (P < 0.01) in the experimental animals. Total cholesterol level in the untreated diabetic group animals were increased significantly 179.6 ± 7.4 (P<0.001) when tested against the control group animals. After thirty days treatment with *WcEe*, significantly decreases total cholesterol level 88.6, ± 6.5 (P < 0.001) in the experimental animals.

The serum MDA (as an indicator of LPO) level in diabetic rats was significantly higher than their control counterparts. Administration of WcEe to diabetic rats significantly decreased the concentration of MDA in serum values than diabetic group (p>0.001). Similar result was also observed in liver homogenate (Figure 1). In the present investigation shows the level of SOD, CAT and GPx in liver of normal rats, diabetic control and treated animals. The liver of the diabetic animals showed reduced level of SOD, CAT and GPx were 3.82 ± 0.8 (P <0.01), 55.2 ± 4.4 (P < 0.01) and 0.09 ± 0.02 (P<0.001) respectively (Table –2).
After thirty days administration with *WcEe* in the diabetic animals SOD, CAT and GPx becomes similar to that of diabetic control 9.5 ± 0.1, 86.2 ± 2.1 and 0.19 ± 0.02 (*P*<0.001). The effects of *WcEe* on liver non enzymic antioxidative parameters in alloxan induced diabetes were shown in Figure 1. Effect of *WcEe* on serum and liver LPO in experimental animals.

Values are mean ± S.E.M., *n* = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey’s Kramer multiple comparison test using GraphPad Instat software **P<0.001, diabetic animals compared with control animals, ##P<0.001 diabetic treated animals compared with diabetic animals.

Figure 2. *WcEe* treatment (2 mg/kg b.wt.) over a period of 30 days exerted a protective effect on the non enzymic antioxidants namely, reduced glutathione (GSH), and nitric oxide (NO).

**Discussion**

The diabetogenic effects of alloxan are notoriously variable during the first 48 hrs after administration. Changes in blood glucose levels are often triphasic consisting of initial hyperglycaemia for 24 to 48 hrs followed by more or less permanent hyperglycaemia (27). The alloxan causes massive reduction in insulin release through the destruction of β cells of the islets of langerhans. Hence the secretion may decrease and leads to increase blood glucose. After administration of *WcEe* drug formulation, stimulates insulin secretion from the remnants β cells or and regenerated β cells. Similar effect i.e. insulinogenic activity with treatment of *Trigonella foenum graecum* (28) and *Mormordica charantia* (29).
Table 2: Effect of WcEe on liver antioxidant enzymes in experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD in liver (Unit/min/mg protein)</th>
<th>CAT in liver (µmoles H₂O₂ decomposed/min/mg protein)</th>
<th>GPx in liver (Unit/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control animals</td>
<td>9.3 ± 0.4</td>
<td>86.5 ± 5.3</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>Diabetic animals</td>
<td>3.82 ± 0.8**</td>
<td>55.2 ± 4.4**</td>
<td>0.09 ± 0.02**</td>
</tr>
<tr>
<td>Diabetic treatment control animals</td>
<td>3.76 ± 0.7</td>
<td>55.0 ± 3.2</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>Diabetic animals treated with WcEe</td>
<td>9.5 ± 0.1##</td>
<td>86.2 ± 2.1##</td>
<td>0.19 ± 0.02##</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey’s Kramer multiple comparison test using GraphPad Instat software **P<0.001, diabetic animals compared with control animals, *P<0.01, ##P<0.001 diabetic treated animals compared with diabetic animals.

Among these parameters, the total protein is fall in the serum of diabetic rats and this result is very similar as previously showed (30). This is due to hypoalbuminemia, which observed in diabetics (31). Hypoalbuminemia is a common problem of animals and is generally attributed to the presence of diabetic nephropathy. However recent findings indicate that synthesis of protein is also diminished in the diabetic rats (32).

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesteridemia (33). Hypercholesteridemia also associated in metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose tolerance (34). The observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis (35).

The present data revealed that persistent hyperglycemia through alloxan generated ROS attack produced marked oxidant impact as evidenced by significant increase in LPO levels of diabetic animals than control non-diabetic animals (both serum and tissue). Previous studies done in diabetic patients and diabetic rats reported same phenomenon (36). Elevated levels of MDA were brought down to the normal values by treatment with WcEe.
Figure 2. Effect of WcEe on liver non enzymic antioxidants in experimental animals

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey’s Kramer multiple comparison test using GraphPad Instat software **P<0.001, diabetic animals compared with control animals ##P<0.001 diabetic treated animals compared with diabetic animals.

In our study MDA (as an indicator of LPO) levels in diabetes group were found to be higher than those in control group, indicating increased free radical generation. Treatment of diabetes with the WcEe on subacute studies caused a decrease in MDA levels. This decrease in MDA levels may increase the activity of catalase (CAT) in rats treated with the extract and hence cause inactivation of LPO reactions (37).

Enzymatic antioxidant such as SOD and CAT are considered primary enzymes since they are involved in the direct elimination of ROS (38). SOD is an important defense enzyme and scavenges O2− anion form H2O2 and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reaction (39).

CAT is a hemoprotein, which catalyzes the reduction of hydrogen peroxides (40). The antioxidant enzymes such as SOD and CAT are known to be inhibited in diabetes mellitus as a result of non-enzymatic glycosylation and oxidation (41). In our study, the activities of SOD and CAT decreased in diabetic rabbits as reported earlier (42) which could be due to inactivation caused by alloxan-generated ROS. The ethanolic extract of Wedelia chinensis had reversed the activities of these enzymatic antioxidants, which might be due to decreased oxidative stress as evidenced by decreased LPO.

Alloxan administration decreased reduced glutathione and nitric oxide production in the liver (Figure 2). These findings are in accordance with Sailaja Devi et al., (2000) (43), which reported relatively low liver and blood NO levels in alloxan-induced diabetes. WcEe administration was
increased liver NO production in the diabetic animals. These findings are very similar to the antioxidant enzymes and lipid peroxidation. This finding strengthens our supposition that antidiabetic and antioxidant effects of WcEe.

In conclusion, our observations have clearly demonstrated that the WcEe exerts remarkable antihyperglycaemic activity due to its possible multiple effects involving mechanism. It has also determined that the WcEe extract possessed a capability to inhibit the lipid peroxidation and activate the antioxidant enzymes and non enzymic antioxidants in diabetes conditions.

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