Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats

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Abstract

Hypoglycemic activity was detected in dichloromethane:methanol extract (1:1) of leaves and twigs of *Catharanthus roseus* (family Apocynaceae), a traditionally used medicinal plant, using streptozotocin (STZ) induced diabetic rat model. Extract at dose 500 mg/kg given orally for 7 and 15 days showed 48.6 and 57.6% hypoglycemic activity, respectively. Prior treatment at the same dose for 30 days provided complete protection against STZ challenge (75 mg/kg i.p. × 1). Enzymic activities of glycogen synthase, glucose 6-phosphate-dehydrogenase, succinate dehydrogenase and malate dehydrogenase were decreased in liver of diabetic animals in comparison to normal and were significantly improved after treatment with extract at dose 500 mg/kg p.o. for 7 days. Results indicate increased metabolization of glucose in treated rats. Increased levels of lipid peroxidation measured as 2-thiobarbituric acid reactive substances (TBARS) indicative of oxidative stress in diabetic rats were also normalized by treatment with the extract. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Antidiabetic activity; *Catharanthus roseus*; Streptozotocin; Glucose metabolism; Glutathione; Lipid peroxidation

1. Introduction

Diabetes mellitus is a metabolic disease as old as mankind and its incidence is considered to be high (4–5%) all over the world (Pickup and Williams, 1997). In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as ‘madhumeha’ and some of them have been experimentally evaluated and the active principles isolated (Chopra et al., 1956; Rajashekharan and Tuli, 1976; Chattopadhyay et al., 1993; Pugazhenthi and Murthy, 1996; Chattopadhyay, 1999; Joy and Kuttan, 1999). *Catharanthus roseus* belonging to family Apocynaceae is known with various names in India and all over the world. Hot water decoction of the leaves and/or the whole plant is used for treatment of diabetes in several countries i.e. Brazil, Cook Islands, Dominica, England, Jamaica, Mozambique, Pakistan, Taiwan, Thailand and West Indies (Don, 1999). In India seven flowers/leaves are used at a time whereas in The Cook Islands 18 leaves boiled in a kettle of water and in The West Indies roots of plants infused in whiskey are used traditionally. Preliminary reports indicate blood glucose lowering activity in alcoholic extract of leaves (Ghosh and Gupta, 1980; Chattopadhyay et al., 1991, 1992). In the present study we have evaluated antidiabetic activity of a dichloromethane–methanol (DCMM) extract of leaves and twigs (L&T) and its effect on enzymes of carbohydrate metabolism to find out possible mechanism of hypoglycemic action. In addition to this the effect of extract was evaluated on glutathione levels, related enzymes and lipid peroxidation as oxidative stress is known to occur in diabetes. Effect of extract on other enzymes of pharmacological importance i.e. as-
partate aminotransferase (AST), alanine aminotransferase (ALT), acid and alkaline phosphatases were also evaluated.

2. Materials and methods

2.1. Plant material

Flowering twigs of *C. roseus* were collected after authentication by Dr. J. Yadava, Defence Agricultural Research Laboratory, Pithoragarh. Voucher specimen of plant sample is available in herbarium file of the institute (HERB/DIP 99/Vinca 13). Collected plant material was washed thoroughly with water and dried in the shade. Dried leaves, twigs and flowers were made into powder in a grinder and were extracted with 10 volumes of dichloromethane:methanol (1:1) by continuous stirring for 48 h. After 48 h the solvent was filtered and remaining parts were re-extracted with the same volume of solvent. Extracts were pooled and evaporated to dryness at 60 °C. The yield of this extract was 17% on dry weight basis. Extracts thus prepared were stored at 4 °C. For preparation of aqueous crude extract, known quantities of wet leaves and twigs were homogenized with distilled water in a mixer grinder and centrifuged at 1000 × g for 15 min, supernatants were freeze dried and used as crude extract (yield 20–24% of wet wt).

2.2. Experimental animals and induction of diabetes

Male Sprague-Dawley rats weighing 250–300 g were used in the present study. Animals were maintained at 22 ± 2 °C with 12 h light and dark cycle, fed on standard pellet diet supplied by Lipton India Ltd. Animals had free access to diet and water. After initial determination of 12 h fasting blood glucose levels (blood drawn from retro orbital plexus) animals were given single i.p. injection of streptozotocin at dose 75 mg/kg (freshly dissolved in physiological saline) and blood glucose was monitored after 24 h and thereafter at weekly intervals after 12 h fasting in case of all the experiments reported here.

2.3. Determination of antidiabetic activity and change in body mass

Animals showing blood glucose levels > 200 mg/dl 48 h after STZ treatment were selected for study. Animals were treated orally for different duration at different doses of test extracts suspended in distilled water. Fasting blood glucose levels were monitored weekly along with untreated controls. Any reduction in blood sugar level in comparison to that of untreated controls was taken as antidiabetic activity. Five animals at a time were used and experiments were repeated twice in identical conditions with same number of control animals making a total of 8–10 animals for each study parameter. Changes in body weight of untreated controls and experimental animals were recorded at the same time, i.e. in fasting state.

2.4. Prophylactic activity against STZ challenge

For determination of prophylactic activity, normal animals were treated with test extracts (aqueous extract and DCMM) at a dose of 500 mg/kg (p.o.) for 30 days. After monitoring blood glucose levels on day 30, animals were challenged to streptozotocin at a dose of 75 mg/kg × 1 (i.p.). Blood glucose levels after 48 h were monitored for induction of hyperglycemia along with untreated control animals (fed with distilled water) challenged with STZ.

2.5. Evaluation of effect on biochemical variables

For in vivo study of effect of DCMM extract on biochemical variables, diabetic animals were treated (*n* = 8-10) with the extract at a dose of 500 mg/kg for 7 consecutive days which showed around 50% hypoglycemic activity. Diabetic animals with similar elevated blood glucose levels and normal animals were kept as diabetic and normal controls respectively (*n* = 8–10). All the three groups were sacrificed by cervical dislocation on day 8 post treatment after fasting overnight. Blood was drawn from the heart. The liver was removed, washed with chilled saline, small weighed portion of the liver were processed for determination of glycogen and glutathione immediately after their removal. Ten percent homogenate (w/v) of liver was prepared in 150 mM KCl using Potter–Elvehjem homogenizer at 4 °C. Two milliliter aliquots of crude liver homogenates were used for assay of lipid peroxidation and rest of the homogenates were centrifuged at 3000 × g for 15 min at 4 °C and supernatants were divided into aliquots and frozen at −20 °C until assayed for different enzymes. Blood plasma was recovered by centrifugation at 1000 g for 10 min at 4 °C.

Effect of extract on activities of different enzyme of glucose metabolism were evaluated *in vitro* using liver homogenate/plasma of normal animals as enzyme source. Extract was dissolved in methanol (10 mg/ml). An aliquot of 10–30 μl as required was added to the assay mixture to give a final concentration of 100 μg/ml. Samples were incubated for 10 min at 37 °C before assay. All assays were performed in triplicate with suitable amount of enzyme and assay mixtures.
containing solvent (methanol) in place of extract served as control.

Blood glucose was estimated by method of Nelson as described by Ashwell (1957). Effect on oral glucose tolerance was evaluated by feeding 10 g/kg glucose after 3.5 h of treatment with 500 mg/kg DCMM extract (Chattopadhyay et al., 1991). Plasma fructosamine was determined by the method of Johnson et al. (1982) with slight modification. In brief, reduction of nitroblue tetrazolium (NBT) (Fig. 1) was monitored at 530 nm using an assay mixture containing 50 μl plasma, 1.75 ml Tris buffer pH 10.6 (pH adjusted with 0.1 N NaOH) and 0.2 ml of NBT (1 mg/ml). Liver glycogen was measured by method of Montgomery (1957). The specific activities of enzymes viz. succinate dehydrogenase (EC 1.3.99.1) (Slater and Bonner, 1952), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), malate dehydrogenase (EC 1.1.1.37) (Shonk and Boxer, 1964), glucokinase (EC 2.7.1.2) (Parry and Walker, 1966), glycogen synthase (EC 2.4.1.11) (Leloir and Goldenberg, 1962), lactate dehydrogenase (EC 1.1.1.27), aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2) (King, 1965), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) (Lowry et al., 1954), γ-glutamyl transpeptidase (EC 2.3.2.2) (Orlowsky and Meister, 1963), glutathione S-transferase (EC 2.5.1.18) (Habig and Jakoby, 1981) were estimated with standard colorimetric and photometric techniques. Glutathione in blood and liver was estimated colorimetrically by using DTNB (Ellman, 1959) and lipid peroxidation in crude homogenates as 2-thiobarbituric acid reactive substances (TBARS) by method of Utley et al., (1967). Protein content of enzyme samples were determined colorimetrically (Lowry et al., 1951).

2.6. Statistical analysis

Values are expressed as mean ± SEM. Unpaired Student t-test was used for statistical comparison. In case of in vivo studies comparison were made between normal and diabetic, diabetic versus diabetic treated animals. Changes were considered significant if the P-value was less than 0.05.

### Table 1

Effect of DCMM extract of C. roseus (leaves and twigs) on blood glucose levels (mg/dl) of STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Dose (mg/kg p.o. × days)</th>
<th>Blood glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control diabetic</td>
</tr>
<tr>
<td>500 × 7</td>
<td>291.36 ± 14.6</td>
</tr>
<tr>
<td>500 × 15</td>
<td>237.98 ± 19.17</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.

* P < 0.001 in comparison with control diabetics.

3. Results

3.1. Antidiabetic and prophylactic activity

Diabetic rats treated with crude aqueous extract at oral dose of 1 g/kg for 21 days showed 20.2% reduction in blood glucose in comparison to untreated diabetic rats. Blood glucose levels of control diabetic and treated animals were 315 ± 15.7, 215.3 ± 25.0 mg/dl respectively (P < 0.05).

DCMM extract showed 57.6 and 48.6% antidiabetic activity (P < 0.001) at dose 500 mg/kg given orally for 15 and 7 days respectively (Table 1). Changes in body weight and nitroblue tetrazolium (NBT) reduction assay a measure of fructosamine in plasma are depicted in Fig. 1. Normal animals treated with extract at dose 500 mg/kg (p.o.) and given oral glucose 10 g/kg showed delay in peak blood glucose levels by 30 min in comparison to untreated rats (Fig. 2).

Prior to treatment for 30 days with aqueous and DCMM extract at dose of 500 mg/kg (p.o.) before STZ challenge provided 27.7 and 100% protection, respectively, in comparison with control animals which received saline daily (Table 2).

3.2. Effect on biochemical variables

3.2.1. Effect on glucose metabolism

In vivo effect of treatment with DCMM extract at dose 500 mg/kg for 5 days on glycogen levels and glucose metabolizing enzymes are given in Tables 3 and 4.

In vitro effect of DCMM extract at conc. 100 μg/ml in assay mixture on enzymic activities are given in Table 5. There was significant stimulation of glucokinase activity whereas other enzymic activities remain almost unaffected.

3.2.2. Effect on glutathione and other biochemical variables

No significant change in glutathione (GSH) levels of diabetic, diabetic DCMM treated animals were observed in comparison to normal animals. Lipid peroxidation was 22.7% more in diabetic animals in comparison with normal animals but was reduced in animals treated with extract at 500 mg/kg (p.o.) × 7 days. Enzymic activities of γ-glutamyl transpeptidase, glutathione S-transferase (GST) and enzymes of toxicological importance, i.e. AST, ALT, acid and alkaline phosphatase activities are given in Table 6.

4. Discussion

For the study of antidiabetic agents, STZ induced hyperglycemia in rodents is considered to be a good
preliminary screening model (Ivorra et al., 1989) and is widely used. STZ, N-[methylnitrocarbamoyl]-D-glucosamine is a potent methylating agent for DNA and acts as nitric oxide donor in pancreatic cells. β cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes (Lukic et al., 1998; Spinas, 1999).

Leaves and flowers of *C. roseus* are used traditionally by diabetic patients in India and are taken as water decoction. Due to this reason crude aqueous extract was given orally at dose of 1 g/kg (generally used oral dose for primary testing of crude products at our laboratory) for a period of 21 days and 20.2% glucose lowering activity was observed. Although the activity is apparently low, it has significance for further studies as STZ treated animals represent chronic model of IDDM and marked destruction of pancreatic structures was observed (slide not shown). Significant hypoglycemic activity was detected in DCMM extract of leaves and twigs (Table 1) along with 100% prophylactic action against STZ challenge during primary screening and need special attention (Table 2). DCMM extract was better tolerated in primary toxicity study and oral LD$_{50}$ was found more than 5000 mg/kg body weight in rats. Other studies on hydroalcoholic extracts of leaves and twigs also indicate fairly higher margin of safety (Chattopadhyay et al., 1992; Chattopadhyay, 1999).

Nitroblue tetrazolium (NBT) reduction assay as a marker of fructosamine levels (protein–ketoamine product) also showed decrease in animals treated with DCMM extract (Fig. 1). This assay is a measure of long term glycemic control (Baker et al., 1985).

The extract was evaluated in vitro as well as in vivo on several biochemical variables. Glycogen levels in liver which were low in diabetic animals, increased several folds in DCMM treated diabetic animals (Table 1). Although glycogen synthetase activity decreased in diabetic animals significantly, the treatment however could not normalize activity. Glycogen content of normal animals in fasting stage was only slightly higher than diabetic animals and this may be due to degradation of glycogen to maintain normal blood glucose levels, whereas glycogen levels in diabetics were found to be very low despite high blood glucose levels possibly due to lower levels of glycogen synthase activity. Accumulation of glycogen in liver of treated animals is somewhat similar to that reported during insulin therapy. When insulin therapy is instituted, hepatic glycogen accumulation begins rapidly and glycogen content rises to 300% of normal levels within 24 h and this inordinate accumulation of glycogen may account for up to 60% of dry liver weight in diabetic animals (Osborn et al., 1953; Spiro et al., 1958; Steiner and King, 1964; Anderson, 1974).

Activity of glucose-6-phosphate dehydrogenase, the first regulatory enzyme of pentose phosphate pathway was found to be decreased in diabetic animals and increased in DCMM treated animals, the activity was higher in comparison to untreated diabetic animals indicating improvement in glucose utilization by this pathway (Table 4).
Fig. 2. Effect of DCMM extract on blood glucose levels of normal rats after feeding glucose (10 g/kg). Values are mean (n = 8), $P < 0.05$ in comparison with control.

Table 2
Prophylactic activity of *C. roseus* extracts against STZ induced diabetes in rats (n = 10)

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mg/dl)</th>
<th>No. of animals diabetic/total</th>
<th>% Animals showing hyper-glycemia</th>
<th>% Protection in comparison to untreated control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated control</td>
<td>74.14 ± 4.05</td>
<td>10/12</td>
<td>83</td>
<td>−</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>85.00 ± 3.11</td>
<td>6/10</td>
<td>60</td>
<td>27.7</td>
</tr>
<tr>
<td>Dichloromethane: methanol extract</td>
<td>70.50 ± 4.81</td>
<td>0/12</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>48 h Post STZ challenge</strong></td>
<td>350.0 ± 10.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated control</td>
<td>91.76 ± 1.77$^a$</td>
<td>0/12</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Animals were treated with extract at dose of 500 mg/kg, p.o. for 30 consecutive days prior to STZ challenge. Values expressed as mean ± SEM (n = 10).

$^a P < 0.001$ in comparison with untreated control group.
Activity of glucokinase, the first regulatory enzyme of glycolytic pathway was also increased by extracts in vitro (Table 5). Decreased activity of glucokinase is reported in diabetes (Storey and Bailey, 1978; Chang et al., 1977). We found no change in lactate dehydrogenase activity in liver of diabetic animals in the present study (Table 5). Increase in lactate dehydrogenase subunit B and isozymes 2,3 and 5 in STZ induced diabetes in Chinese hamsters is reported. Increase in lactate dehydrogenase activity is also reported in hypoglycemic rats (Chang et al., 1977; Lemieux et al., 1984). Malate dehydrogenase plays an important role in the citric acid cycle by providing oxaloacetate for the formation of citrate with acetyl-CoA for generating malate which can feed the cytosolic gluconeogenic pathway (Murray et al., 1998). In the present study, decrease in malate dehydrogenase in liver and plasma of diabetic animals was observed. Levels increased significantly in liver of treated animals whereas in plasma it reached almost normal level. Succinate dehydrogenase activity which was decreased to almost half in diabetic animals was found to be increased in treated animals and levels were even more than normal (Table 5). Increase in succinate and malate dehydrogenase activities in treated animals indicates better utilization of energy yielding intermediates by TCA cycle. These enzymes are reported to be inhibited in tissues of diabetic animals in several studies (Chen and Ianuzzo, 1981; Lemieux et al., 1984; Ianuzzo and Armstrong, 1976). Our results indicate that treat-

Table 3
Effect of DCMM extract of C. roseus on liver glycogen levels and glycogen synthase activity of STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver glycogen (mg/g wet tissue)</th>
<th>Glycogen synthase (µmol UDP formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4.84 ± 0.74</td>
<td>3.01 ± 0.19</td>
</tr>
<tr>
<td>Diabetic</td>
<td>4.37 ± 0.93</td>
<td>0.77 ± 0.18*</td>
</tr>
<tr>
<td>Diabetic treated (500 mg/kg P.O. x 7 days)</td>
<td>22.19 ± 6.69b</td>
<td>1.23 ± 0.19 NS</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 8).

Table 4
Effect of DCMM extract of C. roseus on enzymes of glucose metabolism

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity</th>
<th>Normal</th>
<th>Diabetic</th>
<th>Diabetic treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol NADP+ reduced/min/mg protein)</td>
<td>19.33 ± 1.31</td>
<td>14.32 ± 1.09**</td>
<td>15.46 ± 0.76** NS</td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol pyruvate formed/min/mg protein)</td>
<td>63.16 ± 2.58</td>
<td>65.49 ± 2.84</td>
<td>62.80 ± 5.93</td>
<td></td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol Pot. Ferricyanide reduced/min/mg protein)</td>
<td>4.16 ± 0.40</td>
<td>2.92 ± 0.39*</td>
<td>5.84 ± 0.68††b</td>
<td></td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (µmol NADH oxidized/min/mg protein)</td>
<td>1.56 ± 0.13</td>
<td>1.16 ± 0.07*</td>
<td>3.85 ± 0.21*** †††</td>
<td></td>
</tr>
<tr>
<td>Plasma (µmol NADH oxidized/min/ml)</td>
<td>0.88 ± 0.13</td>
<td>0.52 ± 0.10*</td>
<td>0.80 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 10).

Table 5
In vitro effect of DCMM extract of C. roseus on enzymatic activities of liver

<table>
<thead>
<tr>
<th>Addition</th>
<th>Glycogen synthase</th>
<th>Glucokinase</th>
<th>Lactate dehydrogenase</th>
<th>Succinate dehydrogenase</th>
<th>Malate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control MeOH</td>
<td>4.62 ± 0.37</td>
<td>3.02 ± 0.03</td>
<td>65.74 ± 2.47</td>
<td>4.16 ± 0.49</td>
<td>1.77 ± 0.04</td>
</tr>
<tr>
<td>Extract (100 µg)</td>
<td>5.01 ± 0.29</td>
<td>4.02 ± 0.03*</td>
<td>62.24 ± 2.77</td>
<td>4.73 ± 0.74</td>
<td>1.49 ± 0.17</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 10)

a P < 0.001 in comparison with MeOH control.

b (1) µmol UDP formed/min/mg protein, (2) nmol NADP converted to NADPH/min/mg protein, (3) nmol pyruvate formed/min/mg protein, (4) nmol potassium ferricyanide reduced/min/mg protein, (5) µmol NADH oxidized/min/mg protein.
Table 6
Effect of DCMM extract of C. roseus on glutathione, MDA levels and activities of γ-GT, GST, AST, ALT, acid and alkaline phosphatase

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal</th>
<th>Diabetic</th>
<th>Diabetic treated (500 mg/kg (p.o.) × 7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione (GSH)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood (mg/dl)</td>
<td>108.8 ± 19.7</td>
<td>105.6 ± 9.5</td>
<td>121.5 ± 24.2</td>
</tr>
<tr>
<td>Liver (μg/g wet tissue)</td>
<td>2822.4 ± 186.0</td>
<td>3262.5 ± 292.0</td>
<td>3381.5 ± 544.0</td>
</tr>
<tr>
<td>Lipid peroxidation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (μmol MDA/g wet tissue)</td>
<td>13.70 ± 0.40</td>
<td>16.84 ± 0.20**</td>
<td>33.88 ± 1.40</td>
</tr>
<tr>
<td>γ-Glutamyl transpeptidase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol p-nitroaniline released/min/mg protein)</td>
<td>4.61 ± 0.27</td>
<td>2.76 ± 0.68**</td>
<td>5.75 ± 0.95†</td>
</tr>
<tr>
<td>Plasma (nmol p-nitroaniline released/min/ml)</td>
<td>14.19 ± 1.35</td>
<td>22.34 ± 2.05**</td>
<td>17.58 ± 3.00</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (μmol thioester formed/min/mg protein)</td>
<td>0.59 ± 0.05</td>
<td>0.45 ± 0.06</td>
<td>0.44 ± 0.07</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol pyruvate /min/mg protein)</td>
<td>88.88 ± 7.79</td>
<td>73.47 ± 8.37</td>
<td>78.14 ± 7.03</td>
</tr>
<tr>
<td>Plasma (nmol pyruvate formed/min/ml)</td>
<td>79.18 ± 7.03</td>
<td>163.73 ± 24.25**</td>
<td>107.09 ± 27.21</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol pyruvate /min/mg protein)</td>
<td>84.55 ± 2.22</td>
<td>79.26 ± 8.25</td>
<td>80.33 ± 8.94</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol p-nitrophenol released/min/mg protein)</td>
<td>0.54 ± 0.06</td>
<td>1.06 ± 0.10***</td>
<td>1.17 ± 0.15***</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (nmol p-nitrophenol released/min/mg protein)</td>
<td>9.26 ± 0.16</td>
<td>9.46 ± 0.83</td>
<td>9.98 ± 0.67</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 10)

** P < 0.05, *** P < 0.01, † P < 0.001 in comparison with normal, † P < 0.05 in comparison with diabetic.

ment with DCMM extract of C. roseus increases utili-

Oxidative stress appears to be a key element of the production of secondary complications in diabetes (Wohaieb and Godin, 1987; Godin et al., 1988; Wolff et al., 1991; Thomson and McNeil, 1993; Thornalley et al., 1996). Glutathione, a tripeptide present in millimolar concentrations in all the cells is an important antioxidant (Meister and Anderson, 1983; DeLeve and Kaplowitz, 1991; Lu, 1999). Decreased glutathione levels in diabetes have been considered to be an indicator of increased oxidative stress (Wolff, 1987; McLennan et al., 1991). In the present study not much change was observed in GSH levels either in blood or liver of diabetic animals, however in treated animals GSH levels were marginally high in both blood as well as liver. Lipid peroxidation was found to be increased in liver of diabetic animals which became normal in DCMM treated animals. This indicates that the extract may be helpful in the prevention of damage caused by oxygen free radicals. We have not studied oxidized glutathione levels and this is a limitation of this study.

There was not much change in glutathione S-trans-

ferase activity in diabetic and diabetic treated animals. γ-Glutamyl transpeptidase activity was decreased significantly in liver of diabetic animals and with DCMM extract treatment activity increased (Table 6). γ-Glutamyl transpeptidase has a key role in amino acid transport across membranes and catalyzes the initial step in breakdown of glutathione, i.e. transfer of γ-glutamyl moiety of glutathione to a variety of amino acids and peptides (Meister, 1983). Increase in γ-glutamyl transpeptidase activity in plasma is an indicator of impairment in liver function. In the present study there was an increase in γ-glutamyl transpeptidase activity in plasma of diabetic animals. In C. roseus treated animals activity of this enzyme shows a decrease in plasma and was close to normal activity.

Measurement of enzymic activities of aminotransferases (AST and ALT) and phosphatases (acid and alkaline) is of clinical and toxocological importance as changes in their activities are indicative of tissue damage by toxicants or in disease conditions. AST and ALT activities in liver of diabetic animals remain unchanged in liver though AST activity was little less than normal. Plasma levels of AST were increased around
twice that of normal in diabetic animals and diabetic animals treated with extract show improvement. Recovery of plasma AST levels of diabetic rats towards normal shows that the DCMM extract has no adverse effect on liver functions. Liver alkaline phosphatase activity was found to be significantly increased in diabetic animals. Treatment with extract further caused increase in activity (Table 6). Increase in alkaline phosphatase activity in testes and prostate at 300 mg/kg for 24 days by ethanolic extract of C. roseus leaves is reported in normal animals (Chauhan et al., 1979). Acid phosphatase activity of liver of diabetic rats was also found to be increased. At low dose 75 mg/kg for 24 days ethanolic extract is reported to inhibit acid phosphatase activity and at higher doses i.e. 300 mg/kg stimulation is reported (Chauhan et al., 1979).

Detection of hypoglycemic activity in DCMM extract along with protective effect against STZ challenge and preventive action on lipid peroxidation provides scientific rationale of use of C. roseus as antidiabetic plant. Anti diabetic activity seems to be a result of increase in glucose utilization. Further chromatographic fractionation of the extract may be useful in improving activity and reduction of dose. In preliminary toxicity study it is safe, however chronic toxicity evaluation will be required for human use. Prophylactic activity observed in present study is of much importance and there is a need for further studies on this aspect.

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