EVALUATION OF ANTIDIABETIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITIES OF THE METHANOLIC EXTRACT OF Bauhinia variegata AND Enterolobium cyclocarpum LEAVES IN STREPTOZOTOCIN DIABETIC RATS

Sanad, M. I.; H. H. El-Rafey; R. A. Hassan; and M. A. Taher

ABSTRACT

Hypoglycemic action of methanolic extracts of Bauhinia variegata and Enterolobium cyclocarpum leaves were studied on streptozotocin diabetic rats. Two different doses (200 and 400 mg/kg body weight) of the extracts were given orally to the rats for 22 days. Lipid profile improvement and hepatoprotective effects against liver disorders associated with diabetic complications were also studied. Results revealed that Bauhinia variegata extract showed a strong antidiabetic activity at the two doses. The doses of 400 mg/kg b.wt. showed the highest activity. Enterolobium cyclocarpum extract at the two doses failed to decrease blood glucose level to the healthy range. The mode of Hypoglycemic action of Bauhinia variegata extract may due to the presence of considerable amounts of Flavonoids and polyphenols. The two extracts (Bauhinia variegata and Enterolobium cyclocarpum) could improve lipid profile of diabetic rats and Enterolobium cyclocarpum had weaker ability. Bauhinia variegata methanolic extract resulted in significant decrease in liver malondialdehyde (MDA) and increase in reduced glutathione (GSH).

INTRODUCTION

Bauhinia variegata Linn. (Ceasalpiniaeae) is a medium sized deciduous tree distributed in most tropical countries, including Africa, Asia and South America. Whereas, in Egypt, it is poorly distributed and is used only for landscape purposes. It is traditionally used in herb medicine in treatment of bronchitis, leprosy and tumors. The stem bark is used as astringent, tonic and anthelmintic (Ram and Mehrotra 1980; Ambasta 1998). Infusion of the leaves is used as laxative and for piles, dried buds are used in the treatment of worm infections, tumors, diarrhea and piles (Asima and Satyesh 1992). The stem bark is used in Ayurveda for its antidiabetic activity (Col Herber, 1991). Moreover, the stem bark has been investigated and reported to have antitumor (Rajkapoor et al 2003; Rajkapoor et al 2006), antibacterial, antifungal, antiulcer, and hepatoprotective activities (Bodakhe et al 2007).

Except for Bauhinia variegata, other plants of the genus Bauhinia have been well investigated for their antidiabetic potential. Leaves extracts of these trees demonstrated a hypoglycemic effect (Lemus et al 1999; Silva et al 2002; Lino et al 2004; Fuentes et al 2004). A hypolipidemic action was also stated for Bauhinia sp by Lino et al (2004) in diabetic animals. On the other hand, there were no reports about the effect of Bauhinia sp. extracts on liver disorders induced by reactive oxygen species in diabetic animals.
Enterolobium cyclocarpum tree is a species of the legume genus, Enterolobium. It is a large deciduous canopy tree (though in its native range it is usually evergreen) native to tropical regions of the Americas, from central Mexico south to northern Brazil (Roraima) and Venezuela. It is the national tree of Costa Rica. Immature pods are cooked as a vegetable. The uses of Enterolobium products were summarized by (Orwa et al. 2009). The highly palatable and nutritious pods containing a sugary pulp are consumed readily by livestock. The foliage is also palatable, though to a lesser extent than the pods. The wood of E. cyclocarpum has been found excellent for producing high quality paper. The wood may be used for boat building, because of its durability in water. Tannin from the pods and bark is used in soap making. Bark extracts are used medicinally against colds and bronchitis. Spermicidal, pro-inflammatory, cytotoxicity and polyspecific proteinase inhibitor activities were reported for the plants belonging to the genus Enterolobium (Primorac et al 1985; Neto et al 1991; Mimaki et al 2003; Nakahata et al 2011). There were no publications evaluated the antidiabetic activity of Enterolobium cyclocarpum.

In recent years, a new concept of the antioxidant effects of dietary rich polyphenols sources have emerged, i.e., direct scavenging activity toward reactive species and indirect antioxidant activity. The latter activity is thought to arise primarily via the activation of nuclear factor, which stimulates the activities of antioxidant enzymes, such as glutathione peroxidase, glutathione S-transferase, catalase, quinone oxidoreductase-1, and/or phase II enzymes (Hu 2011). The direct antioxidant activity of dietary polyphenols in vivo is probably limited because of their low concentrations in vivo, except in the gastrointestinal tract where, they are present in high concentrations.

The present study aimed to examine the in vivo biochemical effects of hypoglycemic action of methanolic extracts of both Bauhinia variegata and Enterolobium cyclocarpum leaves at doses of 200 and 400 mg/kg body weight on streptozotocin diabetic rats, and to investigate their hepatoprotective effects against liver disorders induced by reactive oxygen species associated with diabetic complications.

MATERIALS AND METHODS

1- Collection of plant material and extraction

Fresh leaves of Bauhinia variegata and Enterolobium cyclocarpum were collected in April 2010 from the farm of faculty of agriculture, Mansoura university, Egypt. The leaves of both plants were shade dried and coarsely powdered. The powder of both plants were extracted separately by soaking in methanol overnight. The extraction process was repeated twice, then the combined methanol extract was evaporated under vacuum till dryness to obtain greenish brown extract.

2- Determination of flavonoids and polyphenols

Total flavonoid content was determined in both extracts calorimetrically according to (Lin and Tang, 2007). Quercetin was chosen as a standard (the concentration range from 0.005 to 0.1 mg/ml). Flavonoid

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content was expressed as mg quercetin (QE) per g of dry extract. Total polyphenols were determined spectrophotometrically according to Folin-Ciocalteu method as described by Singleton et al (1999) using gallic acid as a standard (the concentration range from 0.025 to 0.5 mg/ml). Polyphenolic content was expressed as mg gallic acid mg / g dry extract.

a) Identification and quantification of flavonoids by HPLC technique.

Flavonoids of *Bauhinia variegata* leaves were separated and quantified by HPLC technique. An Agilent 1100 Series high-performance liquid chromatograph equipped with a diode array detector was used. Column temperature was set at 35 °C, gradient elution was employed with a mobile phase consisting of 50 mM H_{3}PO_{4}, pH 2.5 (solution A) and acetonitrile (solution B). All flavonoids were quantified using the external standard method, and the samples were analyzed in triplicate. The method is described in details by (Mattila et al, 2000).

b) Identification and quantification of polyphenols by HPLC technique

Phenolic compounds were extracted from powder leaves of *Bauhinia variegata* and identified according to the method described by (Goupy et al, 1999). Reversed phase HPLC (RP-HPLC)/diode array detection (DAD) (Hewlett Packard 1050) with a guard column Alltima C18, 5mm (Alltech) was used. A gradient elution was employed using solvent system of A (CH_{3}COOH 2.5%), B (CH_{3}COOH 8%) and C (acetonitrile). The solvent flow rate was 1 ml/min and separation was performed at 35°C. Phenolic compounds were assayed by external standard calibration at 280nm and expressed in mg /g dry matter.

3- Animal experiment

A number of 60 albino rats weighing (100-120g) were kept 7 days for adaptation under laboratory conditions. All rats were fed corn meal diet and allowed free access to water. After adaptation period, seven rats were housed as a healthy group and considered as control (group, 1). The other fifty three rats were fasted for 24 h then injected intraperitoneal by streptozotocin (MP, USA) freshly prepared in 0.1 M citrate buffer , pH 4.5 at a dose of 40mg/kg body weight to induce diabetes mellitus (Ghasemi et al, 2007). In order to stave off the hypoglycemic effect during the first day after streptozotocin injection, rats were given 5% glucose solution orally as reported by (Orhan et al, 2006). After 72 h of streptozotocin injection, serum glucose levels of all diabetic rats fasted for 18 h were determined. Rats showed blood glucose levels over 250 mg/dl were considered as diabetic and were employed in the study. The diabetic rats then randomly divided into 6 groups (7 rats in each). Group (2), represents control diabetic rats, received normal diet for 22 days without any treatment. Group (3), represents diabetic rats, fed a normal diet for 22 days with a metformin hydrochloride powder as a reference drug. Groups (4) and (5) are diabetic rats, received normal diet for 22 days with crude methanol extract of Bauhinia leaves in doses of 200 and 400 mg/kg body weight respectively. Groups (6) and (7), are diabetic rats fed normal diet for 22 days with crude methanol extract of Enterolobium in doses of 200 and 400 mg/kg body weight respectively. Methanolic extracts and reference drug were dissolved in saline solution (sodium chloride, 0.9%)
and given orally by a stomach tube daily; after fasting for 2 hours; for 22 days. Blood samples were taken from orbital plexus in the eyes of rats after 11 and 22 days. The blood samples were centrifuged without anticoagulant at 4000 rpm for 20 min. to separate serum, which kept frozen (-20°C) till analysis. At the end of the experiment (after 22 days), the rats were fasted overnight, killed by decapitation and liver were removed. Liver samples were then prepared for further determinations.

**Biochemical analysis**

Serum total cholesterol (TC) Rosclau et al (1974), triglycerides (TG) Schettler and Nussel (1975), high density lipoprotein cholesterol (HDL-c) Friedewald et al (1972) and glucose Trinder (1969) were estimated using enzymatic kits (Spinreat company, Spain). Serum low density lipoprotein cholesterol (LDL-c) was calculated according to the equation of Friedewald et al (1972).

\[ LDL-c = \text{total cholesterol} - (\text{triglycerides}/5) - \text{HDL-cholesterol} \]

Serum very low density lipoprotein cholesterol (vLDL-c) was calculated according to Norbert (1995) formula:

\[ vLDL-c = (\text{triglycerides}/5) \]

**Antioxidant defence system activity**

The antioxidant defence activities were determined in liver homogenates as follows:

a) **Preparation of liver homogenate**

Liver tissues were perfused prior to dissection with phosphate buffer saline solution pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. The tissues were then homogenized in 5 ml cold phosphate buffer per gram tissue, using tissue homogenizer. The homogenized tissues were centrifuged at 4000 rpm for 15 minutes at 4°C. The resultant supernatant was drawn, and stored on ice till further assay.

b) **Antioxidant activity of liver supernatant**

The resultant liver supernatant was used for the determination of malondialdehyde (MDA) and reduced glutathione (GSH) as antioxidant defence parameters. Glutathione (reduced) and Malondialdehyde levels were estimated by Bio-diagnostic kits according to the methods of Beutler et al (1963) and Satoh (1978), respectively.

**RESULTS AND DISCUSSION**

The present study was conducted to examine the hypoglycemic action of methanolic extract of both Bauhinia variegata and Enterolobium cyclocarpum leaves (at doses of 200 and 400 mg/kg body weight) on streptozotocin diabetic rats. Hepatotrophic effects of the extracts against liver disorders induced by reactive oxygen species associated with diabetic complications were also studied.

**Blood glucose**

Streptozotocin injection at a dose of 40 mg/kg b.wt. caused highly significant increase in blood glucose level. Rats showed blood glucose levels
over 250 mg/dl were considered as diabetic, then randomly divided to 6 groups. Table(1) showed blood glucose levels of the experimental animal groups at zero time (beginning of the experiment), and after 11 and 22 days. Blood glucose of the diabetic untreated rats (group, 2) increased from 384.20 at zero time to 400.72 and 409.22 mg/dl after 11 and 22 days, respectively. This increase was due to the destructive effect of Streptozotocin on β-cells langerhans islets, which lead to insulin deficiency and the absence of available insulin in blood circulation (Vessal et al 2003). The present results agreed with several reports, for instance, Pepato et al (2004) and Volpato et al (2008) illustrated that intravenously streptozotocin administration at a dose around 50 mg/kg b.wt. increased blood glucose to 330 mg/dl or higher.

Table (1): Blood glucose level of different experimental animal groups.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Average blood glucose levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
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<tr>
<td>1</td>
<td>104.00</td>
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<tr>
<td>2</td>
<td>384.20</td>
</tr>
<tr>
<td>3</td>
<td>363.20</td>
</tr>
<tr>
<td>4</td>
<td>361.20</td>
</tr>
<tr>
<td>5</td>
<td>377.00</td>
</tr>
<tr>
<td>6</td>
<td>378.60</td>
</tr>
<tr>
<td>7</td>
<td>382.00</td>
</tr>
</tbody>
</table>

1= healthy rats, 2= diabetic control group (untreated), 3 = reference drug (metformin hydrochloride powder) treated group (500mg/kg b.wt.) ; 4, 5 = Bauhinia variegata extract treated groups (200mg/kg b.wt. and 400mg/kg b.wt., respectively) ; 6, 7 = Enterolobium cyclocarpum extract treated groups (200mg/kg b.wt. and 400mg/kg b.wt., respectively).

It could be noticed from table(1) that blood glucose levels of all treated groups decreased with different extents after 11 and 22 days of oral injection of different extracts. On using the reference drug (group, 3) glucose levels were 363.20 at the beginning of the experiment and decreased to 181.79 and 125.57 mg/dl after 11 and 22 days of drug administration, respectively.

Treatment with Enterolobium cyclocarpum extract (groups 6 and 7) decreased blood glucose levels but they still around the diabetic range (250mg/dl). Moreover, Enterolobium cyclocarpum treated rats with the dose of 400mg/kg b.wt. (group 7) had the lowest potency to decrease blood glucose levels after 11 and 22 days of dose administration. No publications were found dealing with anti-diabetic activity of Enterolobium cyclocarpum preparations.

Obtained data for Bauhinia variegata treated rats (groups 4 and 5) showed a strong anti-diabetic activity, especially at dose of 400mg/kg b.wt. (group 5). Blood glucose values was 377.00 at the beginning of the experiment and decreased to 162.93 and 138.95 mg/dl after 11 and 22 days of dose administration, respectively.

Our findings for the anti-diabetic potency of Bauhinia variegata preparations agreed to a large extent with those obtained by several
researchers, who examined antidiabetic potency for other varieties belonging to the genus of Bauhinia (Lemus et al 1999; Pepato et al 2002; Silva et al 2002; Fuentes et al 2004). On the other hand, the present results were different from those of other researchers, who showed that leaves infusion or alcohol extract from the leaves of B. forficata did not lower glucose blood level in streptozotocin induced diabetic rats (Russo et al 1990; Coimbra-Teixeira et al 1992).

Table(2): Total polyphenols and total Flavonoids of Bauhinia variegata and Enterolobium cyclocarpum leaves

<table>
<thead>
<tr>
<th>Total flavonoids mg quercetin /g dry extract</th>
<th>Total polyphenols mg gallic/g dry extract</th>
<th>extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.30</td>
<td>142.86</td>
<td>Bauhinia variegata</td>
</tr>
<tr>
<td>9.44</td>
<td>29.78</td>
<td>Enterolobium cyclocarpum</td>
</tr>
</tbody>
</table>

Table (2) showed fractionation and identification of flavonoids of Bauhinia variegata leaves by HPLC. Six compounds could be identified, rutin was the predominant flavonoid (86.78mg/100g dry leaves) followed by narengenin, quercetin and rosmarenic (11.21, 4.69 and 4.12 mg/100g dry leaves), respectively. Kaempferol was also present. Recently, rutin, the
predominant detected flavonoid in the present study, was reported to
decrease glucose levels, when administrated as a drug to patients with
diabetes mellitus Sattanathan et al (2011). Rutin is a polyphenolic flavonoid,
which could prompt the intact functional β cells to produce insulin and or
protect the functional β cells from further deterioration (Chakravarthy et al,
1980; Chakravarthy et al, 1983; Hii and Howell, 1985; Vessal et al, 2003;
Coskun et al, 2005; Kamalakkannan and Prince 2006).

**Lipid profile**

Tables (4) and (5) showed blood lipid profile of the experimental
animals. It could be noticed that diabetic control animals (group 2) showed a
significant elevation of total cholesterol, triglycerides, LDL cholesterol, VLDL-
cholesterol, when compared to non diabetic healthy (group 1) rats. Their
values were 140.50 mg/dl, 181.31 mg/dl, 69.91 mg/dl and 36.26 mg/dl,
respectively after 11 days of feeding (table 4). After 22 days of feeding (table
5) they were 147.79 mg/dl, 191.00 mg/dl, 78.12 mg/dl and 38.20 mg/dl,
respectively. Also, diabetic control animals (group 2) showed a significant
decrease in HDL-cholesterol, where their values were 34.33 (mg/dl) and
31.47 (mg/dl) after 11 days and 22 days of feeding, respectively (table 4 and
5).

High atherogenic index AI (3.08) and LDL-c/HDL-c (2.02) ratios were
recorded for the diabetic rats (group 2) after 11 days of feeding. At the end of
feeding period the previously mentioned values were 3.68 and 2.47,
respectively.

These findings agreed with Brixova, (1981), who stated that diabetes
mellitus (especially Type 1) is accompanied by hypercholesterolemia,
hyperlipidemia and hepatic steatosis. Insulin deficiency in diabetes mellitus
leads to abnormal metabolic and regulatory processes, this in turn leads to
accumulation of lipids such as TC and TG in diabetic patients (Goldberg, 1981). Insulin deficiency will lead to decreased activity of lipoprotein lipase and increased mobilisation of free fatty acids from
peripheral fat depots. So the STZ-induced diabetic animal is thus considered
as an animal model of type 1 diabetes mellitus and hyperlipidemia (Suckling
and Jackson, 1993).

Total cholesterol content of diabetic rats treated by all plant extracts
decreased non significantly; comparing to diabetic untreated rats (group 2)
after 11 days of feeding (table 4). Whereas, Bauhinia variegata extract only at
200 or 400 mg/kg b.w showed a significant decrease in blood total cholesterol
compared with the diabetic rat group at the end of the experiment (table 5)
with values of 95.61 and 98.06 mg/dl, respectively.

Concerning serum triglycerides, significant reduction was obtained
only by Bauhinia variegata extract at 200 and 400 mg/kg b.wt., and
metformin hydrochloride reference drug after 11 days of treatment with
values of 158.44, 149.56 and 142.88 mg/dl, respectively. When the
experiment was extended to 22 days, a significant decrease was possessed
by all plant extracts compared with the diabetic rat group (G2), keeping in
mind that Enterolobium cyclocarpum extract had a weaker ability to reduce
serum triglycerides.

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Table (4): Blood lipid profile of different experimental animal groups after 11 days of feeding.

<table>
<thead>
<tr>
<th>LDL/HDL</th>
<th>AI</th>
<th>VLDL-c</th>
<th>LDL-c</th>
<th>HDL-c</th>
<th>TG</th>
<th>TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.22**</td>
<td>0.68*</td>
<td>20.44**</td>
<td>9.43**</td>
<td>43.44**</td>
<td>102.19*</td>
<td>73.31*</td>
</tr>
<tr>
<td>0.22**</td>
<td>3.08**</td>
<td>16.25**</td>
<td>69.91**</td>
<td>34.33</td>
<td>151.31**</td>
<td>140.50**</td>
</tr>
<tr>
<td>0.52**</td>
<td>1.67**</td>
<td>28.58**</td>
<td>35.64</td>
<td>38.03**</td>
<td>142.88*</td>
<td>102.25**</td>
</tr>
<tr>
<td>1.16**</td>
<td>1.92**</td>
<td>31.68**</td>
<td>48.18**</td>
<td>41.83**</td>
<td>158.44**</td>
<td>121.69**</td>
</tr>
<tr>
<td>0.84**</td>
<td>1.46**</td>
<td>29.91**</td>
<td>38.93**</td>
<td>48.12**</td>
<td>149.56**</td>
<td>116.97**</td>
</tr>
<tr>
<td>1.74**</td>
<td>2.66**</td>
<td>34.36**</td>
<td>65.81**</td>
<td>37.77**</td>
<td>171.81**</td>
<td>137.94**</td>
</tr>
<tr>
<td>1.68**</td>
<td>2.53**</td>
<td>34.61**</td>
<td>68.51**</td>
<td>40.49**</td>
<td>173.06**</td>
<td>143.61**</td>
</tr>
<tr>
<td>0.45</td>
<td>0.47</td>
<td>5.71</td>
<td>19.77</td>
<td>9.05</td>
<td>28.57</td>
<td>29.42</td>
</tr>
<tr>
<td>0.61</td>
<td>0.64</td>
<td>7.78</td>
<td>26.92</td>
<td>12.92</td>
<td>38.90</td>
<td>40.05</td>
</tr>
</tbody>
</table>

1 = healthy rats, 2 = diabetic control group (untreated), 3 = reference drug (metformin hydrochloride powder) treated group (500 mg/kg b.w.), 4,5 = Bauhinia variegata extract treated groups (200 mg/kg b.w. and 400 mg/kg b.w., respectively), 6,7 = Enterolobium cyclocarpum extract treated groups (200 mg/kg b.w. and 400 mg/kg b.w., respectively).

Similarly, significant reduction was only recorded for LDL-cholesterol by Bauhinia variegata extract at 200 and 400 mg/kg b.w. and by metformin reference drug compared with the diabetic rat group after 11 days of feeding with values of 48.18, 38.93 and 35.64 mg/dl, respectively. At the end of the experiment, LDL-cholesterol were 29.15, 27.12 and 27.98 mg/kg, respectively. Also, Enterolobium cyclocarpum extract at doses of 200 and 400 mg/kg b.w. significantly decreased serum LDL-cholesterol after 22 days of feeding, where their values reached 57.96 and 51.82 mg/dl, respectively. In other words, Enterolobium cyclocarpum extracts had a weaker ability to reduce serum LDL-cholesterol. At the end of treatment period Bauhinia variegata extract at 200 and 400 mg/kg b.w. significantly reduced serum VLDL-cholesterol to be 25.61 and 24.36 mg/dl, respectively.

The present results were in accordance with those obtained by other researchers. For instance, lino et al. (2004) showed that different extracts of
Bauhinia forficata leaves which were administrated daily to alloxan induced diabetic rats for 7 days at doses of 200 and 400 mg/kg, possessed a significant reduction in plasma glucose, triglycerides and total cholesterol, while LDL-cholesterol content was not altered. Rajani and Ashok (2009) found that alcoholic and aqueous extracts of Bauhinia variegata stem bark effectively decreased plasma cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol and, increased plasma HDL-cholesterol levels in Triton WR1339 (iso-octyl polyoxyethylene phenol) induced hyperlipidemic albino rats. In another study, when high fat diet induced obese rats were administered Bauhinia purpurea methanol extract (200 mg/kg.b.wt.), total cholesterol (TC), triglycerides (TG) and low density lipoproteins (LDL) decreased considerably, while the high density lipoproteins (HDL) increased. The alterations in these lipid profiles were more pronounced with 400 mg/kg.b.wt. (Ramgopal et al., 2010). No available researches dealt with the effects of Enterolobium sp. extracts on lipid profile of diabetic rats.

Lipid profile of animal groups treated with Bauhinia variegata leaves methanol extract, and with reference drug were similar to the healthy non diabetic group. In other words, there were no significant difference between them, meaning that, methanol extract of Bauhinia variegata leaves, like reference drug, were successful in controlling lipid profile of diabetic rats. As mentioned before, Bauhinia variegata leaves in the present study had six different flavonoids. Some of these flavonoids were reported to have inhibitory effect on hepatic cholesterol biosynthesis (Glässer et al., 2002; Lee et al., 2004).

Recently, syringic acid exhibited hepatoprotective and antihyperlipidemic activity against acetaminophen-induced hepatotoxicity rats (Ramachandran, 2010). In the present study, it could be noticed from table (6) that, syringic acid was the most abundant polyphenol (832.62 ppm) in Bauhinia variegata leaves.

Table (6): Polyphenols of Bauhinia variegata leaves identified by HPLC technique.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Phenolic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>178.35</td>
<td>pyrogallol</td>
</tr>
<tr>
<td>12.03</td>
<td>gallic</td>
</tr>
<tr>
<td>30.79</td>
<td>catechol</td>
</tr>
<tr>
<td>34.29</td>
<td>P-OH Benzoic</td>
</tr>
<tr>
<td>43.68</td>
<td>Protocatechuic</td>
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<tr>
<td>286.83</td>
<td>Catechin</td>
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<tr>
<td>462.61</td>
<td>Chlorogenic</td>
</tr>
<tr>
<td>104.07</td>
<td>Caffeic</td>
</tr>
<tr>
<td>832.62</td>
<td>syringic</td>
</tr>
<tr>
<td>97.81</td>
<td>caffeine</td>
</tr>
<tr>
<td>143.13</td>
<td>ferulic</td>
</tr>
<tr>
<td>44.52</td>
<td>p-coumaric</td>
</tr>
<tr>
<td>299.40</td>
<td>Vanillic</td>
</tr>
<tr>
<td>37.64</td>
<td>coumarin</td>
</tr>
</tbody>
</table>
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Moreover, *Bauhinia variegata* leaves had higher polyphenols content than *Enterolobium cyclocarpum* (142.86 and 29.78 mg as gallic acid/g dry extract, respectively, table, 2). So it was suggested that, total polyphenols content of *Bauhinia variegata* especially, syringic acid may contribute the antihyperlipidemic action in diabetic rats.

**Antioxidant defence system activity**

Table (7) showed the effect of administration of methanolic extracts of the investigated plants on MDA and GSH levels in liver tissues of different groups of rats. It could be observed, that, MDA level, the index of lipid peroxidation, significantly increased in liver of streptozotocin diabetic animals; group 2; (49.37 nmol/g tissue) as compared to normal rats; group 1; (21.35 nmol/g tissue). The increase in oxygen free radicals in diabetic animals could be due to impaired glucose metabolism, which leads to oxidative stress (Ceriello et al., 1992). Treatment with *Bauhinia variegata* methanol extract at 200 or 400 mg/kg and reference drug (500 mg/kg) for 22 days resulted in significant decrease in liver tissue MDA. These treatments had liver MDA values of 29.29, 27.22 and 32.57 nmol/g tissue, respectively. On the contrary, *Enterolobium cyclocarpum* extracts at both doses had little effect on liver tissue MDA. In other words, *Enterolobium cyclocarpum* extracts had poor in vivo antioxidant capacity.

**Table (7): Liver MDA and GSH levels of different experimental animal groups after 22 days of feeding.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GSH mg of GSH /g tissue</th>
<th>MDA n mol of MDA formed/g tissue</th>
<th>animal</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.63**</td>
<td>21.35**</td>
<td>1</td>
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<td>2</td>
<td>2.88**</td>
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<td>3.65**</td>
<td>44.89**</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1.18</td>
<td>5.47</td>
<td>0.05 LSD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.61</td>
<td>7.45</td>
<td>0.01 LSD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MDA = malondialdehyde, GSH = reduced glutathione

Also, it was observed that GSH level; which protect the cellular system against toxic effects of lipid peroxidation; was significantly depleted in liver tissue of streptozotocin treated animals; group 2; (2.86 mg/g tissue) as compared to normal rats; group 1; (5.63 mg/g tissue). Treatment with *Bauhinia variegata* methanol extract at 200 or 400 mg/kg and reference drug (500 mg/kg) for 22 days resulted in significant increase in liver tissue GSH. These values were 4.99, 5.36 and 4.73 mg of GSH/g tissue, respectively. *Enterolobium cyclocarpum* methanolic extract treatments (200 or 400 mg/kg b.wt.) for 22 days showed weak effect on GSH levels in liver tissues of experimental rats.
No researches were found dealing with in vivo antioxidant activity of Bauhinia variegata extracts in diabetic rats. So, the present results were supported by Shajiselvin and Muthu (2011) who dealt with Bauhinia purpurea ethyl acetate extract. They showed that administration of this extract to rats fed high fat diet significantly increased the levels of antioxidant enzymes, such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GR) and the level of non enzymatic antioxidant Glutathione (GSH), when compared with high fat diet rats. Also, the same extract lowered the concentration of TBARS, when compared with high fat diet rats. In the present study, the higher polyphenolic content of Bauhinia variegata (142.86mg as gallic acid/g dry extract) than Enterolobium cyclocarpum (29.78mg as gallic acid/g dry extract) may be responsible for the high antioxidant activity (table, 2).

The Antioxidant activity of the polyphenolic flavonoids, is due to their ability to reduce free radical formation and to scavenge free radicals. Most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess a radical-scavenging ability. Both the absorbed flavonoids and their metabolites may display an in vivo antioxidant activity, which is evidenced experimentally by the increase of the plasma antioxidant status, the sparing effect on vitamin E of erythrocyte membranes and low-density lipoproteins, and the preservation of erythrocyte membrane polyunsaturated fatty acids (Pietta 2000).

REFERENCES

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Sanad, M. I. et al.


التأثيرات المضادة لمرض السكر وارتفاع الكبد وراثة لمرض السكر لنساء بسحابات التعبيرية من الوراثة في فئران مyses بدرجة المضادة للأكسدة للمستخلص الميثانوني لأوراق خف الفناء والمعدلة في فئران التجارب المستخدمة لمرض السكر بواسطة استرمابولوسين مصطفى إبراهيم سعد، حلمى حلمى الأفناني، رضوان أحمد حسن و
محمد إبراهيم طاهر
قسم الكيمياء الزراعية – كلية الزراعة – جامعة المنصورة

تم دراسة التأثير الخاض لمرض السكر للمستخلص الميثانوني لأوراق لكلا من نباتان خف الفناء والمعدلة في فئران التجارب المستخدمة بدرجة المضادة للأكسدة للمستخلص الميثانوني لأوراق لكلا من نباتان خف الفناء والمعدلة في فئران التجارب المستخدمة.

استخدمت جرعات بتراكيز 200 مجم مستخلص / كجم وزن حي أو 400 مجم / كجم وزن حي

وأخبرت الفئران بواسطة أقمشة لمدة 22 يوم. كذلك تم دراسة تأثير هذه المستخلصات على مدى تخسيس الكبد ومدى جهادها، كما تم الكشف عن التأثيرات المضادة والمرتبطة بمرض السكر.

وقد أوضحت النتائج المرئية لمستخلصات خف الفناء بقليلات الجمل، حيث تم الكشف عن خصائص مستوي سكر الدم للجسم. وكان أفضل نتائج متصل في المجموعة التي تناولت 400 مجم مستخلص خف الفناء / كجم وزن حي.

كذلك فإن النتائج أوضحت أن المستخلص الميثانوني لأوراق خف الفناء، كما أن الفئيات التي تناولن مستخلص السكر الدم الحديث الرياح لم تنتج لجرعات خفية جرعة تحت المنتجات على كميات معقولة من القلوادات والبولينوليات.

وقد أوضحت النتائج أن كلا المستخلصات لمرض السكر والفئيات المختلفة في الدم، والتحريج قد كانت درجة أن الفئات أقل من خفض الفناء. كذلك أدت جرعات مستخلصات خف الفناء إلى خصائص معروفة في مستوى الملفاتي الدهني.

فأتم تحكم البحث

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