

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

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### **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 400mg/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 be 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. (Ceasalpiniaceae) 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 pills (*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 (*Raj Kapoor et al 2003; Raj Kapoor 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

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<sub>3</sub>PO<sub>4</sub>, 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<sub>3</sub>COOH 2.5%), B (CH<sub>3</sub>COOH 8%) and C (acetonitrile). The solvent flow rate was 1ml/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 ,pH4.5 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 a normal diet for 22 days with crude methanolic 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 methanolic 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 4000rpm for 20 min.to separate serum, which kept frozen (-20C) 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).

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

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

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

### **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 pH7.4 containing 0.16mg/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 4000rpm 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 *Sato* (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. Hepatorotective 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 400.72and 409.22 mg/dl after 11 and 22 days, respectively . This increase was due to the destructive effect of Streptozotocin on  $\beta$ -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 around50 mg/kg b.wt. increased blood glucose to 330 mg/dl or higher .

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

Animal groups	Average blood glucose levels (mg/dl)		
	0 day	11 day	22 day
1	104.00	100.94 <sup>(e)</sup>	103.70 <sup>(d)</sup>
2	384.20	400.72 <sup>(a)</sup>	409.22 <sup>(a)</sup>
3	363.20	181.79 <sup>(d)</sup>	125.57 <sup>(d)</sup>
4	361.20	205.92 <sup>(d)</sup>	156.31 <sup>(c)</sup>
5	377.00	162.93 <sup>(d)</sup>	138.95 <sup>(c)</sup>
6	378.60	248.22 <sup>(bc)</sup>	236.09 <sup>(b)</sup>
7	382.00	290.93 <sup>(b)</sup>	267.53 <sup>(b)</sup>

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 admistration, respectively .

Treatment with *Enterolobium cyclocarpum* extract (groups 6 and7) 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 antidiabetic activity of *Enterolobium cyclocarpum* preparations.

Obtained data for *Bauhinia variegata* treated rats (groups 4 and 5) showed a strong antidiabetic 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 after11 and 22 days of dose administration, respectively .

Our findings for the antidiabetic 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**

Total flavonoids mg quercetin /g dry extract	Total polyphenols mg gallic/g dry extract	extract
14.30	142.86	<i>Bauhinia variegata</i>
9.44	29.78	<i>Enterolobium cyclocarpum</i>

It could be noticed from table (2) that *Bauhinia variegata* methanol extract had higher polyphenols(142.86mg as gallic/g dry extract) and flavonoids (14.30 mg as quercetin /g dry extract) than *Enterolobium cyclocarpum* extract. So, the mode of hypoglycemic action of *Bauhinia variegata* extract may due to the presence of considerable amounts of flavonoids and polyphenols . In this respect, *De-Sousa et al 2004* reported the increase of glucose transport , stimulatory effect of glucose uptake and inhibit intestinal glucose absorption by *Bauhinia* sp flavonoids. The inhabitation of intestinal glucose absorption, as well as glucose-6-phosphatase and hepatic gluconeogenesis by aqueous leaves extract of *B. megalandra* were reported by *Gonzalez-Mujica et al(2003)*. They suggested that this plant might help control hyperglycemia in diabetic patients .These effects appear to be related, at least partially, to the presence of flavonoids, especially quercetin 3-O-alpha (2"-galloyl) rhamnoside and kaempferol 3-O-alpha (2"-galloyl) rhamnoside (*Estrada et al., 2005;Gonzalez-Mujica et al 2005*).

**Table(3): Flavonoids of *Bauhinia variegata* leaves Identified by HPLC technique.**

mg/100g dry leaves	Flavonoids
4.12	Rosmarinic
86.78	Rutin
11.21	narengenin
4.69	quercetin
0.98	Quercetin dehydrate
1.83	Kaempferol

Table (3)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 administered as a drug to patients with diabetes mellitus *Sattanathan et al (2011)*. Rutin is a polyphenolic flavonoid, which could prompt the intact functional  $\beta$  cells to produce insulin and or protect the functional  $\beta$  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, LDLcholesterol,VLDL-cholesterol, when compared to non diabetic healthy (group,1) rats. Their values were 140.50mg/dl, 181.31mg/dl, 69.91mg/dl and 36.26 mg/dl, respectively after 11 days of feeding (table,4). After 22 days of feeding(table 5) they were 147.79mg/dl, 191.00mg/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 98.06mg/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.

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

LDL/HDL	AI	VLDL-c	LDL-c	HDL-c	TG	TC	parameters animal Groups
0.22 <sup>(c)</sup>	0.68 <sup>(d)</sup>	20.44 <sup>(d)</sup>	9.43 <sup>(d)</sup>	43.44 <sup>(ab)</sup>	102.19 <sup>(d)</sup>	73.31 <sup>(c)</sup>	1
2.02 <sup>(a)</sup>	3.08 <sup>(a)</sup>	36.26 <sup>(a)</sup>	69.91 <sup>(a)</sup>	34.33	181.31 <sup>(a)</sup>	140.50 <sup>(d)</sup>	2
0.92 <sup>(b)</sup>	1.67 <sup>(c)</sup>	28.58 <sup>(c)</sup>	35.64 <sup>(c)</sup>	38.03 <sup>(bc)</sup>	142.88 <sup>(c)</sup>	102.25 <sup>(c)</sup>	3
1.16 <sup>(b)</sup>	1.92 <sup>(c)</sup>	31.69 <sup>(abc)</sup>	48.18 <sup>(bc)</sup>	41.83 <sup>(abc)</sup>	158.44 <sup>(bc)</sup>	121.69 <sup>(ab)</sup>	4
0.84 <sup>(b)</sup>	1.46 <sup>(c)</sup>	29.91 <sup>(bc)</sup>	38.93 <sup>(c)</sup>	48.12 <sup>(a)</sup>	149.56 <sup>(c)</sup>	116.97 <sup>(ab)</sup>	5
1.74 <sup>(a)</sup>	2.66 <sup>(ab)</sup>	34.36 <sup>(ab)</sup>	65.81 <sup>(ab)</sup>	37.77 <sup>(bc)</sup>	171.81 <sup>(ab)</sup>	137.94 <sup>(a)</sup>	6
1.68 <sup>(a)</sup>	2.53 <sup>(b)</sup>	34.61 <sup>(ab)</sup>	68.51 <sup>(a)</sup>	40.49 <sup>(abc)</sup>	173.06 <sup>(ab)</sup>	143.61 <sup>(a)</sup>	7
0.45	0.47	5.71	19.77	9.05	28.57	29.42	0.05
0.61	0.64	7.78	26.92	12.32	38.90	40.05	0.01

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) .

Table (5): Blood lipid profile of different experimental animal groups after 22 days of feeding.

LDL/HDL	AI	VLDL-c	LDL-c	HDL-c	TG	TC	parameters animal Groups
0.31 <sup>(c)</sup>	0.76 <sup>(d)</sup>	20.10 <sup>(d)</sup>	14.29 <sup>(c)</sup>	45.04 <sup>(a)</sup>	100.50 <sup>(d)</sup>	79.43 <sup>(c)</sup>	1
2.47 <sup>(a)</sup>	3.68 <sup>(a)</sup>	38.20 <sup>(a)</sup>	78.12 <sup>(a)</sup>	31.47 <sup>(b)</sup>	191.00 <sup>(a)</sup>	147.79 <sup>(a)</sup>	2
0.67 <sup>(c)</sup>	1.23 <sup>(c)</sup>	22.80 <sup>(d)</sup>	27.98 <sup>(c)</sup>	41.02 <sup>(a)</sup>	114.00 <sup>(d)</sup>	91.81 <sup>(c)</sup>	3
0.72 <sup>(c)</sup>	1.34 <sup>(c)</sup>	25.61 <sup>(cd)</sup>	29.15 <sup>(c)</sup>	40.85 <sup>(a)</sup>	128.06 <sup>(cd)</sup>	95.61 <sup>(c)</sup>	4
0.62 <sup>(c)</sup>	1.21 <sup>(c)</sup>	24.36 <sup>(cd)</sup>	27.12 <sup>(c)</sup>	44.33 <sup>(a)</sup>	121.81 <sup>(d)</sup>	98.06 <sup>(c)</sup>	5
1.52 <sup>(b)</sup>	2.33 <sup>(b)</sup>	30.97 <sup>(b)</sup>	57.96 <sup>(b)</sup>	38.26 <sup>(ab)</sup>	154.83 <sup>(b)</sup>	127.18 <sup>(ab)</sup>	6
1.23 <sup>(b)</sup>	1.95 <sup>(b)</sup>	29.98 <sup>(bc)</sup>	51.82 <sup>(b)</sup>	41.74 <sup>(a)</sup>	149.88 <sup>(bc)</sup>	123.54 <sup>(b)</sup>	7
0.41	0.45	5.65	17.05	7.33	28.25	26.39	0.05
0.56	0.61	7.69	23.22	9.98	38.46	35.93	0.01

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) .

Similarly , significant reduction was only recorded for LDL-cholesterol by *Bauhinia variegata* extract at 200 and 400 mg/kg b.wt. 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.wt. significantly decreased serum LDL-cholesterol after 22 days of feeding ,where their values reached to 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 7days 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.**

Concentration (ppm)	Phenolic compounds
178.35	pyrogallol
12.03	gallic
30.79	catechol
34.29	P-OH Benzoic
43.68	Protocatechuic
286.83	Catechin
462.61	Chlorogenic
104.07	Caffeic
832.62	syringic
97.81	caffeine
143.13	ferulic
44.52	p-coumaric
299.40	Vanillic
37.64	coumarin

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 ; group2; (49.37 nmol /g tissue) as compared to normal rats ; group1; (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 400mg/kg and reference drug (500mg/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.**

GSH mg of GSH /g tissue	MDA n mol of MDA formed/g tissue	parameter animal Groups
5.63 <sup>(a)</sup>	21.35 <sup>(c)</sup>	1
2.86 <sup>(d)</sup>	49.37 <sup>(a)</sup>	2
4.73 <sup>(bc)</sup>	32.57 <sup>(c)</sup>	3
4.99 <sup>(b)</sup>	29.29 <sup>(c)</sup>	4
5.36 <sup>(a)</sup>	27.22 <sup>(c)</sup>	5
3.23 <sup>(d)</sup>	43.62 <sup>(b)</sup>	6
3.65 <sup>(cd)</sup>	44.89 <sup>(ab)</sup>	7
1.18	5.47	0.05 LSD
1.61	7.45	0.01

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; ( 2.86mg/g tissue) as compared to normal rats; group 1;(5.63 mg/g tissue). Treatment with *Bauhinia variegata* methanol extract at 200 or 400mg/kg and reference drug (500mg/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 Shajiselin 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).

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التأثيرات المضادة لمرض السكر و ارتفاع الليبيدات والقدرة المضادة للأكسدة  
للمستخلص الميثانولي لأوراق خف الجمل وأذن الفيل فى فئران التجارب  
المستحثة لمرض السكر بواسطة استريبتوزوتوسين  
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تم دراسة التأثير الخافض لسكر الدم للمستخلص الميثانولي لأوراق لكلا من نبات خف الجمل ونبات أذن الفيل فى فئران التجارب المريضة بالسكر بواسطة الحقن بالاستريبتوزوتوسين. استخدمت جرعات بتركيز 200 مجم مستخلص /كجم وزن حى او 400 مجم/كجم وزن حى وأعطيت للفئران بواسطة القم لمدة 22 يوم . كذلك تم دراسة تأثير هذه المستخلصات على مدى تحسن ليبيدات الدم ومدى حمايتها للكبد من التأثيرات المضاعفة والمرتبطة بمرض السكر . وقد اوضحت النتائج القدرة المرتفعة لمستخلصات خف الجمل بكلا الجرعتين على خفض مستوى السكر ، وكان افضل نشاط مقلل لسكر الدم كان لتركيز 400مجم مستخلص خف جمل /كجم وزن حى . كذلك فان النتائج اوضحت ان المستخلص الميثانولي لأوراق أذن الفيل بكلا التركيزين لم تنجح فى خفض مستوى سكر الدم للحد الطبيعي . وقد يفسر التأثير الخافض لمستوى سكر الدم الحادث نتيجة لجرعات خف الجمل على احتواء مستخلصها على كميات معقولة من الفلافونيدات والبولى فينولات عامة . وبالنسبة لليبيدات الدم فقد اوضحت النتائج ان كلا المستخلصين لخفض الجمل واذن الفيل كان لهم دورا فى خفض مستويات الليبيدات المختلفة فى الدم وان كانت قدرة أذن الفيل اقل من خف الجمل . كذلك ادت جرعات مستخلصات خف الجمل الى خفض معنوى فى مستوى المالونداى الدهيد فى الكبد مع حدوث زيادة فى مستوى الجلوتاثيون المختزل .

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