Biochemical Effects of *Cichorium intybus* and *Sonchus oleraceus* Infusions and Esculetin on Streptozotocin-Induced Diabetic Albino Rats

Ahmed, O. M.[1,2*]; Hozayen, W. G. M.[3]; Bastawy, M.[3]; Hamed, M. Z.[3]

[1]Division of physiology, Zoology Department, Faculty of Science, Beni-Suef University, Egypt.
[2]Faculty of Oral and Dental Medicine, Nahda University, Beni-Suef, Egypt
[3]Biochemistry Division, Chemistry Department, Faculty of Science, Beni-Suef University, Egypt.

[*]osamamoha@yahoo.com

Abstract: This study was designed to assess the effect of oral administration of *Cichorium intybus* and *Sonchus oleraceus* infusions at the dose level of 100mg/kg b. wt and esculetin at the dose level of 6mg/kg b. wt for 4 weeks on the impaired oral glucose tolerance, insulin secretory response, serum lipid profile and oxidative stress in streptozotocin-induced diabetic rats. The treatment of diabetic rats with *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin resulted in a marked amelioration of the impaired glucose tolerance at all examined periods after oral glucose loading and the lowered insulin and C-peptide levels. The impoverished liver glycogen content and elevated liver glucose-6-phosphatase and serum AST and ALT activities of fasting diabetic rats were profoundly corrected as result of treatment with plant infusion and esculetin. Also, these treatments lead to improvement in serum lipid profile indicated by that decrease in serum total lipid, total cholesterol, triglyceride, LDL-cholesterol and vLDL-cholesterol levels and increase in HDL-cholesterol level. The antioxidant defense system was potentially improved in diabetic rats as a result of treatments. The hepatic lipid peroxidation was profoundly decreased and the total thiol and glutathione concentrations were detectably increased. In conclusion, the treatment of diabetic rats with *Cichorium intybus* and *Sonchus oleraceus* infusions and their active constituent, esculetin improved the diabetic state and antioxidant defense system; esculetin seemed to the most effective. However, further clinical studies are required to assess the efficacy and safety of these treatments in diabetic human beings.
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Keywords: Experimental diabetes mellitus; Streptozotocin; *Cichorium intybus; Sonchus oleraceus;* esculetin.

1. Introduction

Diabetes is one of the major diseases of the industrialized and non-industrialized societies (WHO, 1980, 1992; American Diabetes Association, 2002; Cefalu, 2006). Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries (Maiti *et al.*, 2004). It is estimated that 25% of the world population is affected by this disease (Maiti *et al.*, 2004). Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (Maiti *et al.*, 2004). It is a serious metabolic disorder with micro- and macrovascular complications that results in significant morbidity and mortality as recorded by Kamalakkannan and Prince at 2006. The increasing number of aging populations, consumption of calorie rich diets, obesity and sedentary life style have lead to a tremendous increase in the number of patients with diabetes World wide (Simpson *et al.*, 2003). Hyperglycemia is involved in the etiology of development of diabetic complications (Hongxiang *et al.*, 2009). Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver (Hongxiang *et al.*, 2009). Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations (Wadkar *et al.*, 2008).

Cichorium intybus, also known as chicory, common chicory, succory and wild succory, is a wild plant from the class, Dicotyledones, family Compositae and genus *Cichorium* and grows in many parts of the world (Kocsis *et al.*, 2003). Chicory is one of the commercially cultivated transgenic crops authorized by government of the US (Wang and Cui, 2010). Chicory used as important medicinal herb, has been used in folk medicine for liver disorders, gallstones and inflammations of the urinary tract since the 17th century (Kocsis *et al.*, 2003). In addition to an important sources of inulin, fructans, polyphenols and cichoric acid (Milala *et al.*, 2009; Zhang *et al.*, 2005) in food industry, natural products extracting from chicory, which contains saccharides, organic acid, polyphenol (Heimler *et al.*, 2009; Yang,

2009), alkaloid, triterpenes, sesquiterpenes, coumarins, flavone (Ablimit *et al*., 2008; Mao *et al.*, 2009; Wu and Luo, 2009; Yang *et al*., 2009). It is well-known from the literature that main active compounds of chicory are: inulin, fructooligosaccarides, caffeic acid derivatives, flavonoids and polyphenols (Kocsis *et al*., 2003).

Sonchus oleraceus, belong to family Compositae, has rough, thorny petioles that embrace the main stem (Yin *et al*., 2007). It blossoms yellow flowers from May to September, and the seeds ripen in July. The seeds are used for medicine and young leaves are edible (Yin *et al*., 2007). It was suggested that the amount of polyphenol and the antioxidant activity in plants depends on environmental factors such as growing season (Howard *et al*., 2002) and location (Ma *et al*., 2003). An infusion was reported to be used to bring on a tardy menstruation and to treat diarrhea (Moerman, 1998). The latex in the sap is used in the treatment of warts (Duke and Ayensu, 1985). It is also said to have anticancer activity (Duke and Ayensu, 1985).

Esculetin (6, 7-dihydroxy coumarin), isolated from stem bark of *Fraxinus ornus* or *Fraxinus rhynchophylla* and also derived from the herbs *Cichorium intybus*, *Artemisia Capillaris, Artemisia scoparia* and *Bougainvillea spectabillis*, has antioxidant, anti atherosclerotic anti inflammatory and analgesic activity (Tubaro *et al*, 1988; Gilani *et al*, 1998; Martin-Aragon *et a*l, 1998; Holland *et al*., 2000; Miao-Jane *et al*., 2002; Pan *et al*., 2003; Huang *et al*; 2004). Also, esculetin has been shown to increase glucose uptake and plasma-membrane glucose transporter type 1 (GLUT-1) content in vascular smooth –muscle cells (VSMC) exposed to 5 and 23 mM glucose in cultures (Alpert *et al.*, 2002) and suppressive activity on oxidative damage to DNA (Tahara *et al*., 2005).

More recently esculetin has been reported to inhibit oxidative damage induced by *tert*-butyl hydroperoxide in rat liver (Lin *et al*., 2000). Esculetin efficiently attenuated the oxidative stress-induced cell damage *via* its antioxidant properties (Kim *et al*., 2008).

MATERIALS AND METHODS

Experimental animals:

Male albino rats (*Rattus norvegicus*) were used as experimental animals in this investigation. They were obtained from the animal house of Research Institute of Ophthalmology, Giza, Egypt. The experimental animals were male albino rats of about 3 months old and weighing about 130 – 190 g. Animals were given water and were supplied daily with excess amount of pellets of known weight and composition as a standard diet. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. All animal procedures follow and are in accordance with the recommendations for the proper care and use of laboratory animals (Canadian Council on Animal Care, CCAC, 1993).

Collection of plants and preparation of infusions:

Cichorium intybus and *Sonchus oleraceus* leaves were collected from cultivated regions of Beni-Suef Nile Delta Valley during period of February and March 2010. They were authenticated by Dr. Mohamed Ahmed Fadl lecturer of taxonomy, Botany Department, Faculty of Science, Beni-Suef University, Egypt. The leaves were cleaned, dried in shade and powdered by electric grinder. The aqueous extracts of each plant in the form of infusion was prepared according to the method of Swanston-Flatt *et al.* (1990). The powdered leaves were added to the already boiling distilled water and infused for 15 minutes. Then, the infusion (2% w/v) was filtered and the filtrate was freshly used for oral administration to the diabetic rats at dose level of 100 mg/kg b. wt for 4 weeks according to Jamshidzadeh *et al*. (2006) and Ahmed (2010).

Esculetin:

Esuletin, the active constituent of *Cichorium intybus* and *Sonchus oleraceus***,** was obtained from Sigma Chemical Company, USA. It was dissolved in distilled water and given by oral administration at dose level of 6 mg/kg b. wt for 4 weeks according to Gilani *et al*. (1998) and Ahmed (2010).

Animals grouping and induction of diabetes:

Diabetes mellitus was induced in the overnight fasted experimental animals by a single intraperitioneal injection of streptozotocin (STZ) (45 mg/kg b. wt) (Sigma Chemical Company, USA), dissolved in citrate buffer (PH 4.5) (EL-Seifi *et al*., 1993 and Ahmed, 2001).

Ten days after streptozotocin injection, rats were deprived of food over night (10 – 12), blood samples were taken from lateral tail vein after 2 h of oral glucose administration (3 g/kg b.wt) and serum glucose concentration was measured. Rats with a 2-hour serum glucose level ranging from 180 – 300 mg/dl were considered mildly diabetic and included in the experiment.

The considered rats were divided into five groups as follows:

Group I (Normal group)

Rats of this group were given distilled water (5 ml/kg b. wt) by gastric intubation for 4 weeks.

Group II (Diabetic control):

Rats of this group were regarded as a diabetic control group and given distilled water (5 ml/kg b. wt) by gastric intubation for 4 weeks.

Group III (Diabetic treated with *Cichorium*

***intybus* infusion):**

Rats of this group were treated daily with *cichorum intybus* infusion given orally at the dose level of 100 mg/ kg b. wt by gastric intubation daily for 4 weeks.

Group IV (Diabetic treated with *Sonchus oleraceus* infusion):

Rats of this group were treated daily with *sonchus oleraceus* infsuion given orally at the dose level of 100 mg/kg b. wt by gastric intubation daily for 4 weeks.

Group V (Diabetic treated with esculetin):

Rats of this group were orally administered esculetin at a dose level of 6 mg/kg b. wt (dissolved in ml distilled water) by gastric intubation daily for 4 weeks.

By the end of experimental period, oral glucose tolerance test was performed and in the text day, all animals were overnight fasted and sacrificed.

Blood sampling:

After 4 weeks of treatment, blood samples were obtained from lateral tail vein of overnight fasted rats at 0, 1, 2 and 3 hours after oral glucose loading to perform oral glucose tolerance test. In the next day, all animals were sacrificed and blood samples were obtained from jugular vein. Blood samples were left to clot and then centrifuged at 3000 rpm for 15 minutes and supernatant serum was separated. The obtained sera were kept in deep freezer at -30°C till used for biochemical analysis.

Oral glucose tolerance test (OGTT):

This test was performed for normal, diabetic, and diabetic treated rats at the end of the 4th week of treatment with *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin administration.

Successive blood samples were then taken at 0, 1, 2, and 3 hours following the administration of glucose solution (3 g/kg b. wt) through gastric intubation. Blood samples were left to clot and centrifuged. Serum was obtained for determination of glucose concentration.

Biochemical Examination:

Glucose concentration was determined according to method of Trinder *et al.* (1969) using reagent kits purchased from Spinreact Company (Spain). Liver glycogen content was determined according to the method of Seifter *et al. (*1950). Serum insulin and C-peptide levels were determined in Diabetic Endocrine Metabolic Pediatric Unit (DEMPU), Center for Social and preventive Medicine, New children Hospital, Faculty of Medicine, Cairo University using radioimmunoassay kits of DPC (Diagnostic Products Corporation, Los Angeles, USA) [coat-A-count] according to the method of Marschner *et al*. (1974) and Beyer *et al.* (1979), respectively.

Serum total lipids concentration was determined according to the method of Frings *et al.* (1972) using reagent kit purchased from Diamond Diagnostics Chemical Company (Egypt). Serum cholesterol concentration was estimated according to the method of Allain *et al.* (1974) using reagent kits purchased from Reactivos Spinreact Company (Spain). Serum triglyceride concentration was determined according to the method of Fossati and Prencipe (1982) using reagent kit purchased obtained from Reactivos Spinreact Company Spain. Serum HDL-cholesterol concentration was measured according to the method of Allain *et al.* (1974) using reagent kit obtained from Reactivos Spinreact Company, Spain. Serum LDL-cholesterol concentration was determined according to Friendewald *et al.* (1972) formula:

LDL – cholesterol = total cholesterol – triglycerides/5 - HDL-cholesterol

Serum vLDL-cholesterol was calculated according to Norbert (1995) formula:

vLDL-cholesterol conc. = triglycerides / 5

Cardiovascular index was determined according to Ross (1992) formula:

CVR = total cholesterol conc. / HDL-cholesterol conc.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity in serum was determined according to the method of Bergmeyer *et al*. (1978) by using reagent kits purchased from Diamond Diagnostic (Egypt).

Liver lipid peroxidation was determined according the method of Preuss *et al*. (1998). Liver glutathione content was determined according to the procedure of Beutler *et al*. (1963). Liver total thiol content was determined according the method of Koster *et al*. (1986)**.**

Statistical analysis:

The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985) followed by LSD test to compare various groups with each other. Results were expressed as mean ± standard error (SE). For each variable, the F-probability indicates the significance between groups in general.

3. Results

Biochemical changes:

As illustrated in figure 1, the glucose tolerance curves of normal, diabetic control and diabetic treated rats reached their peaks at 1 hour after glucose loading, and then decreased gradually as the time

extended to 3 hours. The OGTT curve of diabetic rats exhibited an enormous elevation as compared with that of normal ones. The serum glucose concentration of diabetic rats was highly significantly increased (P<0.01; LSD) at all points of OGTT as compared with their corresponding normal values. The oral administration of *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin for 4 weeks produced a potential amelioration (P<0.01) of the elevated values. Esculetin followed by *Cichorium intybus* infusion seemed to be more effective than *Sonchus oleraceus* infusion.

As presented in figures 2 ad 3, the serum insulin and C-peptide concentrations were profoundly decreased (P<0.01) in the diabetic rats. The treatments of the diabetic rats with the tested agents resulted in a detectable increase of the decreased values. However, the treatment with *Sonchus oleraceus* infusion produced a significant increase of serum insulin level; the treatment with esculetin induced a significant increase of serum peptide level.

Table 1 shows the effect of treatment on liver glycogen content and various enzymes related to carbohydrate metabolism in the diabetic rats. The impoverished liver glycogen content of the diabetic rats was highly significantly (P<0.01; LSD) increased after treatments with *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin. The activities of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in serum and glucose-6-phosphatase (G-6-Pase) in liver of diabetic rats were highly significantly elevated (P<0.01). After treatments, these elevated activities of diabetic rats were highly significantly reduced (P<0.01; LSD). The esculetin seemed to be most effective in decreasing liver glucose-6-phosphatase and serum ALT activity while chicory appeared to be the most potent in suppressing the elevated AST activity.

Concerning lipid profile (Table 2), The diabetic control rats exhibited marked elevations of serum total lipid, total cholesterol, triglyceride, low density lipoprotein (LDL)-cholesterol and very low density lipoprotein (vLDL)-cholesterol levels. The serum high density lipoprotein (HDL)-cholesterol concentration, on the other hand, was significantly (P<0.05) reduced in the diabetic control animals as compared with the normal ones. The LDL-cholesterol /HDL-cholesterol and total cholesterol /HDL-cholesterol ratios (table 3), as indices of cardiovascular risk, were profoundly increased in the diabetic rats. The treatments with *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin produced a remarkable amelioration of all these variables of lipid profile to various extents. Esculetin appeared to be the most effective hypolipidemic agent.

Concerning antioxidant defense system and oxidative stress (table 4), the diabetic rats exhibited a highly significant (P<0.01) decrease in glutathione and total thiol contents, and increase in lipid peroxidation. On the other hand, diabetic rats treated with all tested materials exhibited noticeable amendment of the antioxidants defense system. Moreover, esculetin appeared to be the most effective on glutathione, *Sonchus oleraceus* (sonchus) infusion appeared to be the most effective on total thiol and *Chicorium intybus* is the most effective in decreasing lipid peroxidation.

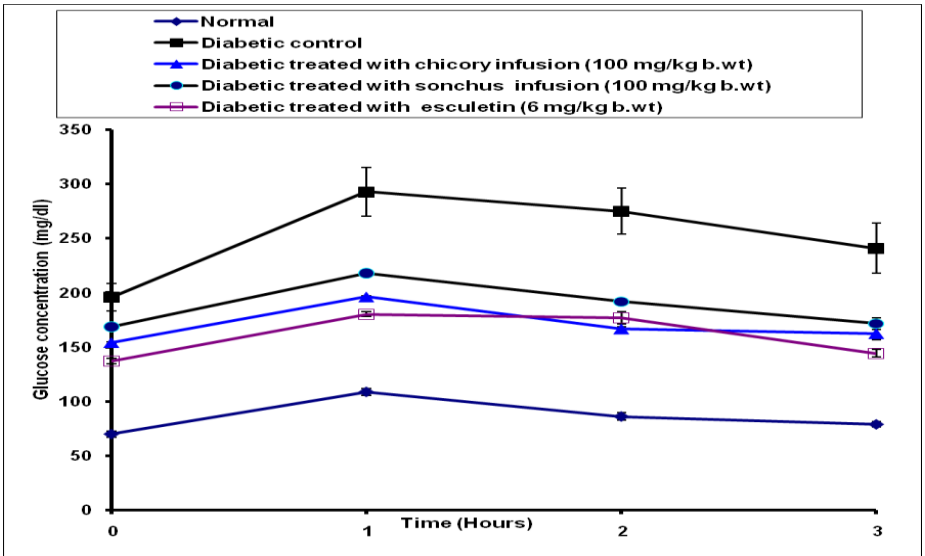

Figure 1: Effect of chicory and sonchus infusions and esculetin administration on oral glucose tolerance test (OGTT) in streptozotocin-induced diabetic rats.

F –probability: p < 0.001; LSD at the 5% level: 16.49; LSD at the 1% level: 22.2

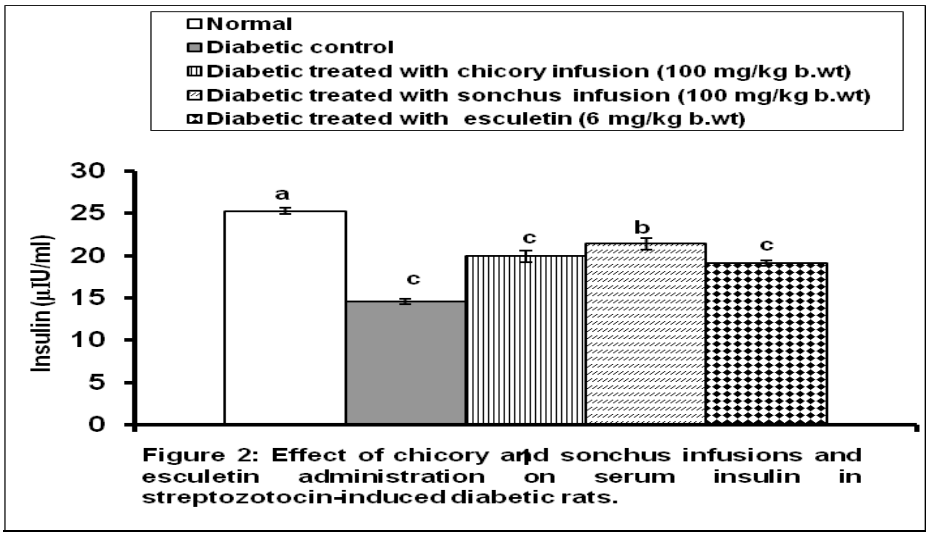

Figure 2: Effect of chicory and sonchus infusions and esculetin administration on serum insulin in streptozotocin-induced diabetic rats.

F-Probability: P<0.001; LSD at 5% level: 1.47, LSD at the 1% level: 1.99

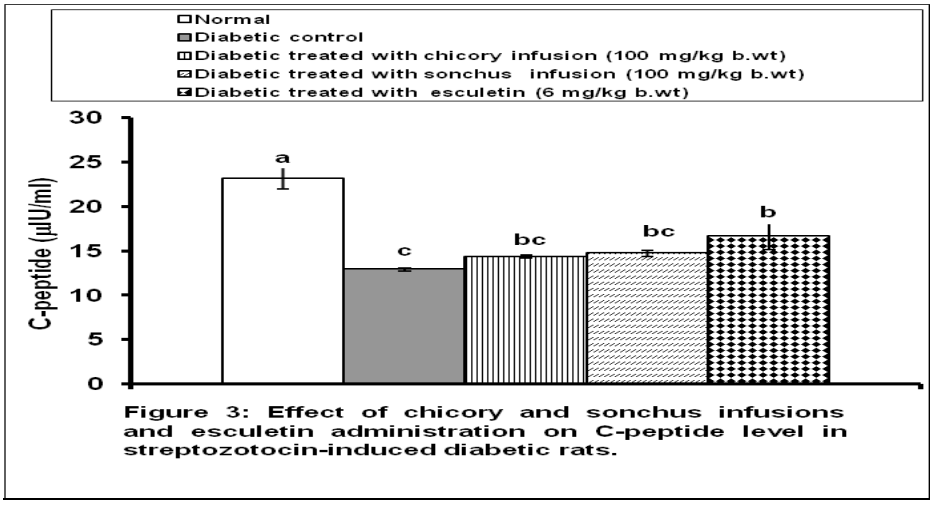

Figure 3: Effect of chicory and sonchus infusions and esculetin administration on C-peptide level in streptozotocin-induced diabetic rats.

F-Probability: P<0.001; LSD at the 5% level: 2.58; LSD at the 1% level: 3.49

Table 1: Effect of chicory and sonchus infusions and esculetin administration on liver glycogen content and liver glucose-6-phosphatase activity as well as serum AST and ALT activities in streptozotocin-induced diabetic albino rats.

Parameter / Group	Liver glycogen (mg/g tissue)	Liver Glucose-6-phosphatase (mU/g tissue)	Serum AST (U/L)	Serum ALT (U/L)
Normal	19.8 ±0.52^{a}	30.74 ±2.55^{c}	14.41 ± 0.44^{b}	13.58± 0.21^{c}
Diabetic control	2.06±0.12^{c}	299.24±22.32^{a}	65.11± 2.52^{a}	50.16± 0.84a
Diabetic treated with chicory infusion (100mg/kg b. wt)	5.28±0.21^{b}	182.33±12.51^{b}	16.08± 0.51^{b}	20.16± 0.44^{b}
Diabetic treated with sonchus infusion (100mg/kg b. wt)	7.71±0.52^{b}	160.21±11.31^{b}	24.61 ±1.22^{b}	23.41 ± 0.99^{b}
Diabetic treated with esculetin (6mg/kg b. wt)	6.66±0.31^{b}	59.19±11.42^{c}	25.51 ± 0.51^{b}	9.01 ± 1.01^{ab}
F-probability	P < 0.001	P < 0.001	P < 0.001	P < 0.001
LSD at the 5%level	2.82	16.71	9.61	5.49
LSD at the 1%level	3.82	2.88	13.01	7.44

Means, which share the same superscript symbol(s) are not significantly different.

Table 2: Effect of chicory and sonchus infusions and esculetin administration on serum lipid profile in streptozotocin-induced diabetic rats.

Group \ parameter	Total lipid (g/l)	Total cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Triglyceride (mg/dl)	vLDL-cholesterol (mg/dl)
Normal	6.45±0.71^{c}	131.61±2.11^{d}	82.41±2.11^{d}	38.61±0.461^{a}	53.11±0.71^{d}	10.58± 0.11^{d}
Diabetic control	13.57±0.12^{a}	283.11±1.91^{a}	222.11±2.22^{a}	22.73± 0.60^{c}	190.12±1.11^{a}	38.11± 0.21^{a}
Diabetic treated with chicory infusion (100mg/kg b. wt)	8.27± 0.11^{b}	196.97±2.90^{b}	157.91±2.90^{b}	24.07± 3.81^{bc}	71.55±0.61^{bc}	14.55± 0.11^{c}
Diabetic treated with sonchus infusion (100mg/kg b. wt)	8.18±0.11^{b}	183.58±3.71^{bc}	140.13±3.81^{bc}	27.07± 0.91^{bc}	81.93±0.71^{b}	16.37± 0.11^{b}
Diabetic treated with esculetin (6mg/kg.b.wt)	7.03±0.12^{c}	167.33±1.48^{c}	125.03±1.81^{c}	29.33± 0.51^{b}	68.17±2.80^{c}	12.55± 0.20^{d}
F-probability	P< 0.001	P < 0.001	P< 0.001	P < 0.001	P < 0.001	p< 0.001
LSD at the 5% level	0.82	18.36	19.38	19.38	10.54	1.34
LSD at the 1% level	1.11	24.85	26.22	26.22	14.26	1.82

Means, which share the same superscript symbol(s) are not significantly different.

Table 3: Effect of chicory and sonchus infusions and esculetin administration on cardiovascular risk indices of streptozotocin-induced diabetic rats:

Group \ parameter	Total cholesterol/HDL-cholesterol	LDL-cholesterol/HDL-cholesterol
Normal	3.42 ± 0.11^{d}	2.12 ± 0.11^{d}
Diabetic control	12.79 ± 1.01^{a}	10.02 ± 0.91^{a}
Diabetic treated with chicory infusion (100mg/kg b. wt)	8.33 ± 0.80^{b}	6.18 ± 0.51^{b}
Diabetic treated with sonchus infusion (100mg/kg b. wt)	7.07 ± 0.81^{bc}	4.83 ± 0.61^{bc}
Diabetic treated with esculetin (6mg/kg b.wt)	5.83 ± 0.31^{c}	3.97 ± 0.31^{c}
F-probability	P < 0.001	P < 0.001
LSD at the 5% level	2.12	1.66
LSD at the 1% level	2.87	2.24

Means, which share the same superscript symbol(s) are not significantly different.

Table 4: Effect of chicory and sonchus infusions and esculetin administration on liver lipid peroxidation and glutathione and total thiol content of streptozotocin diabetic albino rats.

Group \ parameter	Lipid peroxidation (nmol MDA/100mg tissue)	Glutathione (nmol/100mg tissue)	Total thiol (nmol/100 mg tissue)
Normal	29.65±0.21^{c}	105.01±0.71^{a}	335.21±12.21^{a}
Diabetic control	48.41±0.51^{a}	58.11±1.31^{d}	108.81±2.11^{d}
Diabetic treated with chicory infusion (100mg/kg b. wt)	31.21±0.31^{c}	73.36±2.01^{c}	238.25±3.11^{c}
Diabetic treated with sonchus infusion (100mg/kg b. wt)	32.46±0.51^{c}	93.11±0.31^{b}	302.11±8.91^{ab}
Diabetic treated with esculetin (6mg/kg b. wt)	42.11 ±0.61^{b}	96.55±0.91^{ab}	280.75±6.41^{bc}
F- probability	P < 0.001	P < 0.001	P < 0.001
LSD at the 5% level	3.70	8.68	54.02
LSD at the 1% level	5.11	11.75	73.08

Means, which share the same superscript symbol l(s) are not significantly different.

Histological changes of islets of Langerhans:

The pancreas is made up of an acinar exocrine component and a diffuse exocrine one, which constitutes the pancreatic islets or islets of Langethans. The exocrine portion consists of lobules which in turn consist of pancreatic acini that are tightly packed together with very little connective tissue in between and their constituent acinar cells appear roughly triangular in outline (Fig. 4A).

In normal animals, the endocrine portion of pancreas, islets of Langerhans, is scattered through out as irregular and spheroidal masses of pale stained cells with rich vascular supply. In the islets, cells are arranged in irregular cords between which are capillaries (Fig. 4B).

Modified aldehyde fuchsin method

demonstrates three main types of cells, alpha (a), beta (b) and delta (d) cells. All are irregular, granular and polygonal cells with central spherical nuclei beta (b) cells are the most abundant cells found in the endocrine pancreas, occupying the core of the islets and contain numerous granules. Alpha (a) and delta (d) cells form the periphery of the islets. Delta cells are usually located adjacent to alpha cells and are somewhat larger in size (Fig. 4A).

Following streptozotocin administration, at a dose level of 45 mg/kg. b. wt., subtle alterations in the pancreatic islets cells were observed (Fig. 5) and the architecture of the normal islets was disrupted. The islet cells of the diabetic control showed vacuolated cytoplasm. Many necrotic cells are visible. Many nuclei appear irregular and many cells showed extreme hydropic degeneration (Fig. 5).

On the other hand, the treatment with *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin administration stimulates recovery of islets (Figs. 6, 7 and 8). At the end of the experiment, the islets regain their normal architecture with fewer hydropic vacuolated cells in the treated groups as compared with the diabetic control rats. Alpha and delta cells appeared more intact and are granulated. There are still few necrotic areas (n) and vacuolations (v).

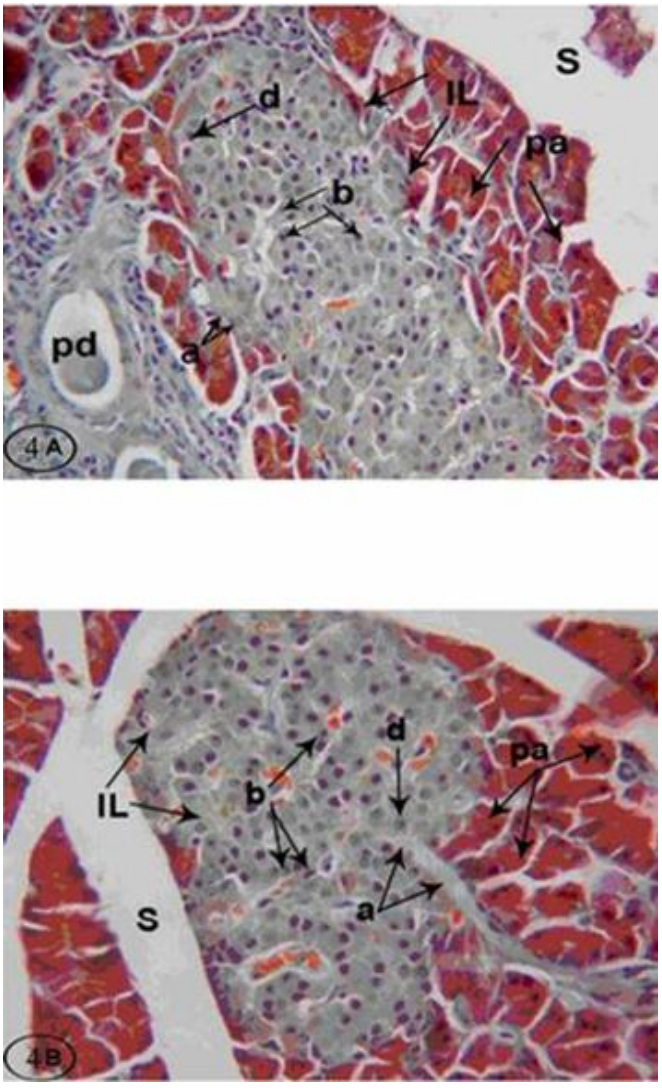

Fig. 4: Pancreata of normal male albino rats. The pancreas is subdivided by septa (s) into pancreatic lobules. The exocrine portion of the pancreas consists of pancreatic acini (pa) while endocrine portion consists of the islets of Langerhans (IL) which are scattered throughout the pancreas and contain alpha cells (a), beta cells (b) and delta cells (d). Pancreatic duct (pd) is also observed (Fig. 4A and B). X 400

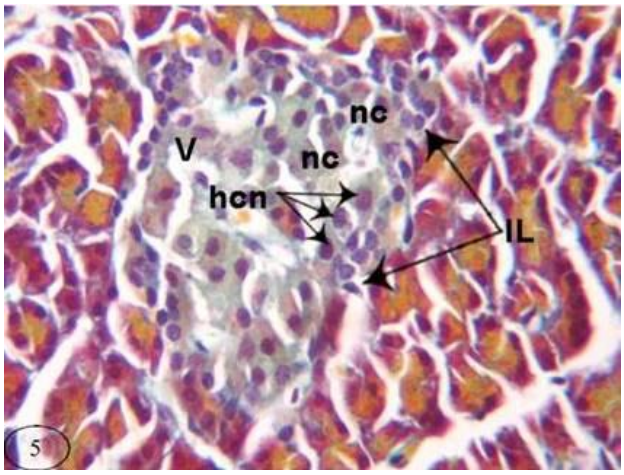

Fig. 5: Pancreata of diabetic rats. Normal architecture of the islets is disrupted. Islets of Langerhans (IL) exhibited hydropic cells, necrotic cells (n), vacuolations (v) and irregular hyperchromatic nuclei (hcn). X 400

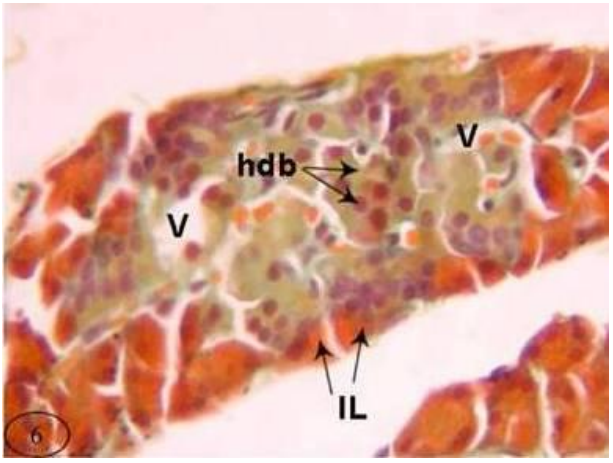

Fig. 6: Pancreata of diabetic rats treated with *Cichorium intybus* (100 mg/kg b. wt). There are still few vacuolations (v) and highly divided of β-cells in the islets of Langerhans (IL). X400

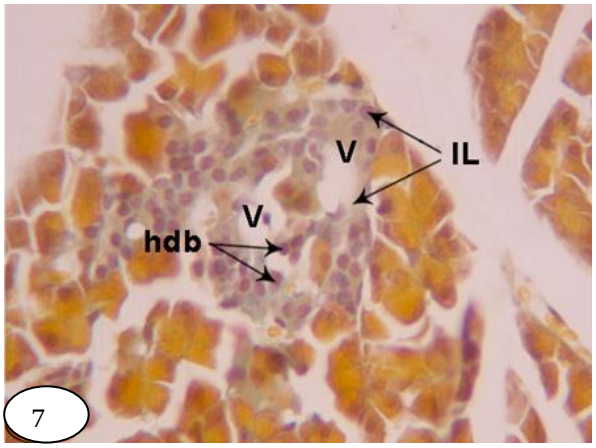

Fig. 7: Pancreata of diabetic rats treated with *Sonchus oleraceus* (100 mg/kg b. wt). There are still few vacuolations (v) and highly divided of β-cells in the islets of Langerhans (IL). X400

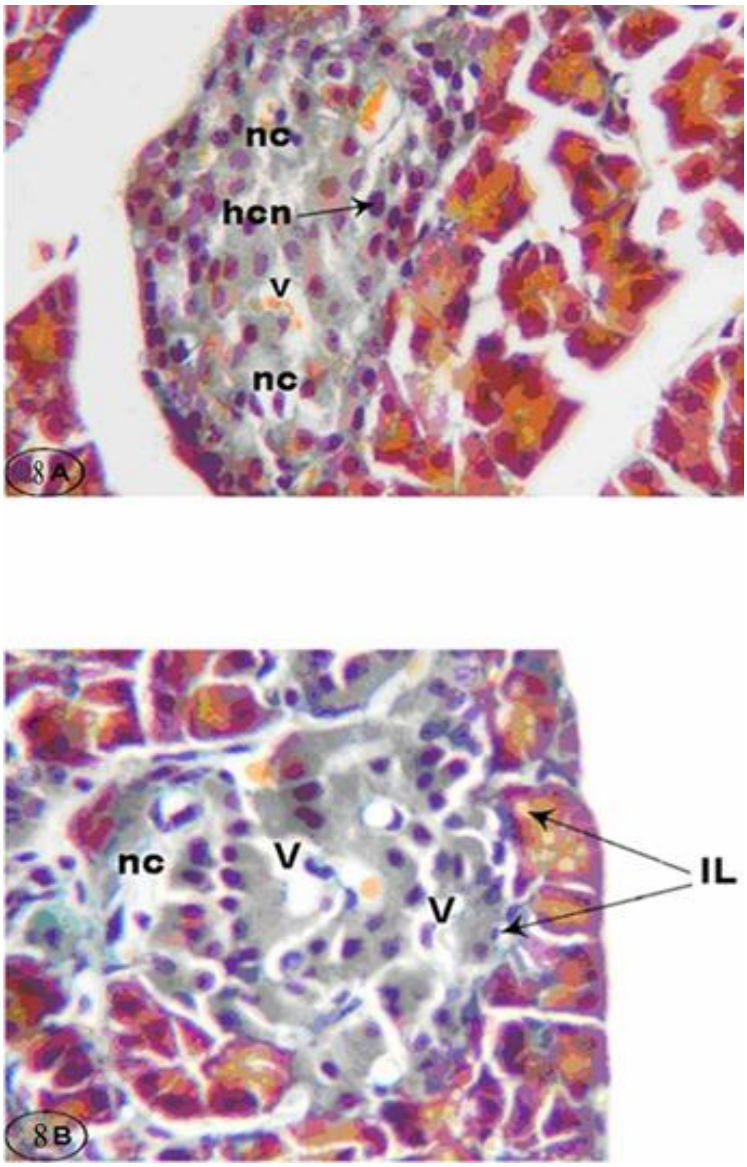

Fig. 8: Pancreata of diabetic rats treated with esculetin (6 mg/kg b. wt). There are still few necrotic areas (nc), vaculations (v), irregular hyperchormatic nuclei (hcn) in the islets of Langerhans (IL) (Fig. 8A and B). X400

4. Discussion

Oral glucose tolerance test (OGTT) is a well-accepted and frequently used assay to screen the antihyperglycemic activity of any hypoglycemic agent (Alberti and Zimmet, 1998). In the diabetic animals, the present data indicate a marked increase in serum glucose levels as compared to normal rats. These results run parallel with Akhani *et al.* (2004), Ahmed (2005), Ahmed (2006) and Ahmed *et al*, (2006). According to Kamal (1991), glucose intolerance could arise from either a defect in insulin secretion as in case of insulin dependent diabetes mellitus or a defect in insulin resistance as in case of non-insulin dependent diabetes mellitus. Diabetogenic agents, like STZ, selectively destruct β-cells of the islets of Langerhans in the pancreas (Abdel-Moneim *et al.*, 2001; Ahmed, 2009) leading to an inhibition of the insulin synthesis and elevation of blood glucose due to (a) a reduced entry of glucose to peripheral tissues, muscle and adipose tissues (Beck-Nielsen *et al*, 1994; Beck-Nielsen, 2002), (b) increased glycogen breakdown (Gold, 1970) and (c) increased gluconeogensis and hepatic glucose production (Raju *et al.*, 2001). Insulin resistance in NIDDM causes elevation in blood glucose due to the same reasons (Powers, 2005). The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they

have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar *et al.*, 2008). The present data demonstrated that the treatment of diabetic rats with either *Cichorium intybus* or *Sonchus oleraceus* caused a potential amelioration of glucose tolerance. Decrease in the elevated serum glucose levels is in agreement with the results of Kim and Shin, (1998). The present study also agrees with the results of Kennedy and Merimee (1981), Baker *et al.* (1984) and Donnelly, (1996) and Pushparaj *et al.* (2007) who attribted the hypoglycemic effect of *Cichorium intybus* may be due to the presence of flavonoids.

The present study revealed a highly significant decrease in fasting insulin level of streptozotocin diabetic rats. This finding agrees with Akhani *et al*. (2004) and Ahmed (2009) and may be ascribed to the diabetogenic effect of STZ which leads to destruction and decreased number of β-cells in the islets of Langerhans as indicated in the present study. Serum insulin concentration was increased markedly as a result of treating diabetic rats with esculetin and *Cichorium intybus.* The present study revealed a highly significant decrease in fasting serum C-peptide level of streptozotocin-induced diabetic rats. Treatment with esculetin which is found in the plants *Cichorium intybus* and *Sonchus oleraceus* potentially increased the levels of insulin and C-peptide; these results are in accordance with those of Steiner (2004).

Liver glycogen level may also be considered as a marker for assessing anti-hyperglycemic activity of any drug (Grover *et al.,* 2000). The present study indicated marked depletion of liver glycogen content associated with maked increase of glucose-6-phosphatase activity. These results are in accordance with those of Ahmed (2005), Ahmed *et al*. (2006) and Ahmed (2010) who found that STZ-induced diabetes reduced hepatic glycogen content and increased glucose-6-phosphatase activity in diabetic rats. These results are also in agreement with the work of Grover *et al.* (2000). The elevation of liver glycogen content in the present investigation after treatment with *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin may be due to amelioration of the glycogen metabolic enzyme activities secondary to the increase of insulin levels in the blood. Consistent with the lowering effect of *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin on elevated hepatic glucose-6-phosphatase activity of diabetic rats, diabetes-induced elevation in the activities of other gluconeogenic enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were potentially improved as a result of treatment. As reported by Maiti *et al.* (2004) and Khan *et al.* (2010), the elevation in serum AST and ALT activities in diabetic rats, could be related to excessive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores. In accordance with the present study, Upur *et al.* (2009) mentioned that *Cichorium intybus* extract reduced serum AST, ALT activity. The higher hepatic AST and ALT levels in the diabetic rats in the current study are thought to be consistent with their greater need for gluconeogenic substrates (Abdel-Moneim *et al.*, 2001; Ahmed, 2001 and 2005). The decrease of hepatic transaminases activity with treatments, on the other hand, may reflect less demands gluconeogenesis (Rawi *et al.*, 1998; Ahmed, 2001; Ahmed *et al.*, 2006). Our results demonstrated that hepatic glucose-6-phosphatase activity in diabetic rats was significantly higher than that of normal rats and administration of *Cichorium intybus* infusion markedly lowered its activity. In concurrent with the present data, Pushparaj *et al.* (2001) and Pushparaj *et al.* (2007) demonstrated that daily administration of *Cichorium intybus* (C1E) (125 mg/kg) for 14 days to diabetic rats reduced serum glucose by 20% and hepatic glucose-6-phosphatase activity.

In view of the lipid profile, the diabetic rats exhibited marked elevation of serum total lipid, triglyceride, total cholesterol, LDL-cholesterol and vLDL-cholesterol concentrations. The ratios of LDL-cholesterol to HDL-cholesterol and total cholesterol to HDL-cholesterol (cardiovascular risk indices) were also profoundly raised in the diabetic rats. These results are in agreement with the findings of Mathe (1995), Ulicna *et al.* (1996), Wasan *et al.* (1998) and Abdel-Hamid *et al.* (2007) who recorded marked increases in serum triglyceride and cholesterol levels in alloxan and STZ diabetic animals and in agreement with Rawi (1995), Abdel-Moneim *et al.* (2001, 2004) and Ahmed (2006) who found increased levels of LDL-cholesterol, vLDL-cholesterol and raised LDL-cholesterol to HDL-cholesterol ratios in poorly controlled diabetic patients and non-treated diabetic rats. HDL-cholesterol, on the other hand, revealed a different behavioral pattern where it was detectably lowered in the diabetic rats. This agrees well with observation of Osman and Kandil (1991) and Abdel-Moneim *et al*. (2004) who demonstrated a marked decrease of this variable in IDDM patients and streptozotocin diabetic rats and disagree with Rawi (1995) who revealed a very highly significant increase in alloxan diabetic rats. In the present study, treatments of streptozotocin diabetic rats with esculetin and *Cichorium intybus* and *Sonchus oleraceus* infusions produced potential improvement of the altered serum lipid variables. These results agree with Kim and Shin (1998) which demonstrated

that the potent hypercholesterolemic and hypotriglyceridemic effects of *Cichorium intybus* could be due to the presence of inulin. The ratios of LDL-cholesterol to HDL-cholesterol and total cholesterol to HDL-cholesterol, representing the cardiovascular risk indices were significantly improved in the diabetic treated rats. These results go parallel with many studies. Ramirez-Tortosa *et al.* (1999), and Miao-Jane *et al*. (2002) reported that esculetin prevent atherosclerosis effectively by decreasing the susceptibility of LDL particles to oxidative modification.

Earlier investigations have reported that the extract of *Cichorium intybus* has antidiabetic and hypolipidemic activities in STZ-induced diabetic rats (Pushparaj *et al.*, 2007).

In view of oxidative stress, the present results indicated that streptozotocin-induced diabetes damages the β-cells of the islets of Langerhans also hepatocytes, nephrons and cardiomyocytes (Selvan *et al.*, 2008). STZ induces oxidative stress, which results from enhanced free radical formation and/or defects in antioxidants defense causes severe tissue damage and may lead to number of diseases like coronary artery disease, atherosclerosis, cancer and diabetes (Chakraborty and Das, 2010) and produced marked elevation of liver lipid peroxidation and detectable reduction of liver total thiol and glutathione content (GSH). These data are consistent with those of several authors. Derouich and Boutayeb (2002), Abdel-Moneim *et al.* (2002), Ahmed (2003) and Adewole *et al.* (2007) reported that abnormalities of antioxidant defense systems have been demonstrated in both experimentally induced diabetes and in patients with diabetes mellitus. Ihara *et al.* (1999) examined oxidative stress markers in diabetic rats and found increased reactive oxygen species (ROS) in pancreatic islets. Diabetic and experimental animal models exhibit an increase in the oxidative stress due to persistent and chronic hyperglycemia and depletion in the activity of the antioxidative defense system and thus promote free radical generation (Baynes and Thorpe, 1997).

Treatment of diabetic rats with *Sonchus oleraceus* and *Cichorium intybus* infusions and their active constituent, esculetin attenuated oxidative stress and improved rat antioxidant defense system in the liver. These results are in according with Komali *et al.* (1999) and Moller *et al.* (1999) who revealed that the significant hydroxyle radical scavenging activity of the *Sonchus oleraceus* is due to phenolic compounds present in its extracts. Also, *Cichorium intybus*, a member of asteraceae, is one of the important medicinal plants because of the presence of inulin, coumarins, flavonoids and vitamins (Duke, 1983). Among coumarins, esculin, esculetin, cichoriin and umbeliferone have been reported in chicory (Evans, 1996; Bais *et al.*, 1999; Rehman *et al.*, 2003). *Sonchus oleraceus* and *Cichorium intybus* infusions and esculetin exhibited antioxidant activity which may play a crucial role to diminish and/or prevent oxidative damage produced by STZ.

In conclusion, this study calls the attention to the need of further biochemical investigation of the plants constituents and invites collaboration in the development of clinical field studies to asses the efficacy of the herbalist use of medicinal plants in treatment of diabetes. Our results proved that *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin administration improved glucose tolerance, serum insulin levels and metabolic pathways as well as oxidative stress and antioxidant defense system. Esculetin seemed to be more potent than the *Cichorium intybus* and *Sonchus oleraceus* infusions in affecting most of these aspects. However, further clinical studies are required to assess the benefits and safety of *Cichorium intybus* and *Sonchus oleraceus* infusions and esculetin before using these agents as drugs in human beings.

Corresponding author
Ahmed, O. M
Division of physiology, Zoology Department, Faculty of Science, Beni-Suef University, Egypt.
osamamoha@yahoo.com

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