

The effects of wild cherry & cumin on erythromycin-induced hepatic inflammation in diabetic rats. Biochemical, histological & histochemical study

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Summary:

Background: Complications of diabetes have become more common as the rate of diabetes increased. This complication includes liver and diabetic autonomic neuropathy. Erythromycin is generally well tolerated, but the high dose causes side effects (liver dysfunction). Herbs have been used as food and for medicinal purposes for centuries. There are many herbal remedies suggested for diabetes and diabetic complications, for example cumin, wild cherry and berries. This study was carried out to investigate the most effective extracted from approved selected antidiabetic plants on control blood glucose, lipid profile level and protective effect against oxidative stress in diabetic rats treated with erythromycin.

Objective: The aim of the present investigations is to examine histological, histochemical and biochemical studies the effect of wild cherry & cumin on the liver & kidney tissues.

Materials and methods: Fifty six Wister male rats were used and divided into two groups: Group I animal controls (N), Group II diabetic group treated with alloxan (D) which divided into 5 subgroups: I (DW) treated with wild cherry, II (DC) treated with cumin, III (DE) diabetic animals treated with erythromycin, IV (DEW) animals treated with wild cherry oil, and V (DEC) animals treated with cumin.

Results: Liver tissue of diabetes group revealed necrotic and vacuolated cells, dilated and congested portal vessels as well as areas of inflammatory cell infiltration in D&DE groups, while in DEW&DEC groups hepatocyte architecture appears more or less like control. Kidney tissue of D&DE animals obvious mesangial expansion and basement membrane thickening. While DEW&DEC groups showed no significant differences than D&DE groups.

Conclusions: Wild cherry and cumin can be recommended as a support for the prevention of diabetic complications for liver tissue, but not for kidney tissue.

Key words: Liver & Kidney tissues, Diabetes mellitus, erythromycin, wild cherry, cumin.

J Fac Med Baghdad
2011; Vol. 53, No. 4
Received May 2011
Accepted June 2011

Introduction:

Herbs have been used as food and for medicinal purposes for centuries. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. The use of medicinal herbs has focused on various herbs that possess anti-platelets, anti-tumor or immune stimulating properties that may be useful adjunct in reducing the risk of disease and treatments (1). Diabetes and Herbs: Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. Patients with type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication. Though pathophysiology of diabetes remains to be fully understood, experimental evidences suggest the involvement

of free radicals in the pathogenesis of diabetes, and more importantly in the development of diabetic complications. Free radicals are capable of damaging cellular molecules, DNA, proteins and lipids leading to altered cellular functions (2). Botanical products can improve glucose, lipid metabolism and antioxidant status (3). The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated. There are many herbal remedies suggested for diabetes and diabetic complications, for example *Gymnema*, garlic, *Trigonella foenum graecum* (fenugreek), *Mangifera indica* (Mango), *Phyllanthus amarus* (bhuiawala), *Eugenia jambolana* (Indian gooseberry, jamun) (4), cumin (5), wild cherry (6), essential oil of onion (*Allium cepa* L.) (7) Cinnamon (8) and berries (9). Herbal treatments are becoming increasingly popular as the herbal preparations have no or least side effects.

Erythromycin: Erythromycin (C37H67NO13) was studied because of its widespread use in humans as a broad-spectrum macrolide antibiotic (slows the growth or kills sensitive

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bacteria) by reducing the production of important proteins needed by the bacteria to survive (10). Erythromycin diffuses readily into most body fluids. In the absence of meningeal inflammation, low concentrations are normally achieved in the cerebrospinal fluid but the passage of the drug across the blood-brain barrier increases in meningitis. In the presence of normal hepatic function, erythromycin is concentrated in the liver and is excreted with bile (11).

Wild Cherry:

Genetic name:cherry

Latin name: Prunus serotina

Family name: Rosaceae

Wild cherry bark has a long history of uses as an ingredient in cough syrups and is used particularly for dry, nonproductive, and irritating coughs and pleurisy. It is also beneficial for nervous dyspepsia and lack of appetite. Wild cherry contains prunasin, a cyanogenic glycoside, which gives it its antispasmodic and sedative properties, but might make it toxic in large amounts or when taken for longer than several weeks (6). Cherries contain anthocyanins (the red pigment in berries) which have been shown to reduce pain and inflammation in rats. Anthocyanins are also potent antioxidants under active research for a variety of potential health benefits (12).

Cumin:

Latin name: Cuminum cyminum

English name: Cumin

Is a pale green in color and elliptical in shape with deep furrows, it is a hot, nutty flavored spice (5). Cumin seed was once widely used for food flavoring in Europe, it is much employed in India, and it is used as a flavor in cakes and bread. Cumin is an aromatic, astringent herb that benefits the digestion and acts as stimulant to the sexual organs and improves liver function (13). The seed is antispasmodic, carminative, galactagogue. The seed contains 2.5% essential oil, it is used for flavoring beverages, and the other compounds are monoterpenes, sesquiterpenes, aromatic aldehydes and aromatic oxides. And small amount of terpenes, terpenals, terpenones, and terpen esters (14).

Materials and Methods:

Fifty six Wister male rats weighing 150-200 gm (3 - 6 months old) were used in this study. The animals were supplied by the Breeding Center / College of Medicine / University of Baghdad , given standard food and had free access to water, they were also maintained under standard conditions of humidity, temperature and 12 hr light / cycle. Rats were starved, and then they were injected intraperitoneally with a freshly prepared solution of alloxan monohydrate dissolved in normal saline in a single dose of 100 mg / kg body weight over a period of 10 minutes (15). Because alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution orally after 6 hr for the next 24 hr to prevent hypoglycemia (16). Animals were randomly assigned into 2 groups as shown in the following table I: Group I contained 8 animals, all of

them were injected with sterilized buffer alone & regarded as the normal control group. The remaining rats (48 in number) were the diabetic rats treated with alloxan, they were regarded as the experimental group II which is further divided into 5 subgroups, 8 animals for each.

Table I: Animal groups

Groups	Subgroups	Notes
Normal Control	N (normal)	Given buffer only
Experimental	D (diabetic)	Given alloxan
	DW (diabetic + wild cherry)	Given alloxan + wild cherry
	DC (diabetic + cumin)	Given alloxan + cumin
	DE (diabetic + erythromycin)	Given alloxan + erythromycin
	DEW (diabetic + erythromycin + wild cherry)	Given alloxan + erythromycin + wild cherry
	DEC (diabetic + erythromycin + cumin)	Given alloxan + erythromycin + cumin

5 days after alloxan injection (to make sure that diabetes was induced), diabetic animals were given erythromycin (250 mg/ kg/ orally twice daily) for 10 days (to ensure induction of liver inflammation) (17), then treated with herbs, wild cherry and cumin. Each group was subdivided into 4 groups, each to be sacrificed 14, 21, 28, 35 days after diabetes induction. Rats were fasted for 12 hours prior to sacrificing. Animals anesthetized by using Nembutal solution (0.06 ml/gm body weight) (S.S.N. A. LaBallasler, 33501 Libourne cedex France), blood samples were collected, serum was separated for biochemical studies, then Liver & kidney samples were obtained for histological and histochemical studies. Samples were fixed in 10% buffered formalin for 24 hrs, washed with tap water, dehydrated by graded ethyl alcohol (50%, 70%, 80%, 90% & 100% twice), cleared in xylene, embedded in paraffin wax for 2 hrs (3 changes), blocked and sectioned into 4-5 μ thick sections. After deparaffinization, some of the formalin-fixed sections were stained using hematoxylin and eosin, to be examine under light microscope. Periodic acid-Schiff method was used in other sections for visualization of the polysaccharide material, sections then mounted with DPX, and prepared for examination with the light microscope. Changes in blood glucose levels & lipid profiles among these groups were compared statistically by means of one way analysis of variance (ANOVA) test. P-value less than 0.05 considered to be significant.

Results:

Biochemical results:

Blood glucose of D&DE rats was increased significantly as compared to control animals. A reduction was observed in

blood glucose in D & DE rats treated with essential oil of wild cherry and cumin (DEW&DEC). (Table II). Table III showed an increase in serum lipid and triglyceride concentration in D&DE groups, while those which treated with herbs revealed decrease in their conc.

Table II: Changes in blood glucose during the study period in N, D, DE, DEW, DEC groups

groups	Glucose(mg/dl)14 days	Glucose (mg/dl) 21 days	Glucose (mg/dl) 28 days	Glucose(mg/dl) 35 days
N	85-1.6*	95-3.0*	90-1.1*	95-2.0*
D	300-3.5*	290-3.0	285-2.0*	280-3.4*
DE	320-2.0*	300 - 3.2	280-2.3*	280-2.5*
DEW	170-2.5*	150-3.6*	155-2.5*	150-2.5
DEC	160-2.0	150-2.5*	170-4.0*	160-1.5*

*Values are mean $\pm$ SEM, P < 0.05

Table III: Chang in lipid profile in different studied groups

Groups	Total serum lipid (mg/dl)	Serum triglycerides (mg/dl)
N	98.5 $\pm$ 2.1*	97.5 $\pm$ 2.5*
D	140 $\pm$ 3.2*	154 $\pm$ 6.2*
DE	150 $\pm$ 3.3*	155 $\pm$ 2.5*
DEW	116.6 $\pm$ 3.3*	107 $\pm$ 4.5*
DEC	112.6 $\pm$ 5.2*	110 $\pm$ 5.4*

*Values are mean $\pm$ SEM, P < 0.05.

Histopathological results: Liver: The liver of control rats appeared to be divided into the classical hepatic lobules; each is formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The cell cords were separated by narrow blood sinusoids (Fig. 1-A). The histopathological examination of diabetic rats (D) showed focal necrosis of the hepatocytes associated with inflammatory infiltration (Fig. 1-B). Other hepatocytes appeared more or less as normal. The livers also, showed dilated and congested portal vessels as well as areas of inflammatory cell infiltration in DE group (Fig. 1- C).Herbs-treated liver architecture appeared more or less similar to control and in some part of the tissue the activated cells (cell division) could be noticed, with the exception of some hemorrhagic areas in the blood sinusoid (Fig. 1-D, E). **Kidney:** Examination of the kidney of the control rats revealed normal glomeruli with thin glomerular basement membranes, normal cellularity and patent capsular space surrounding by proximal and distal ducts (Fig. 2-A).Light microscopy of the kidney sections from D and DE rats revealed atrophy of some glomeruli , while in others showed an increase in the thickness of mesangial basement membrane , increase in the matrix of the glomeruli and hyalinization of the arterioles (Fig. 2-B). Herb administrated to diabetic animals showed no effect on glomeruli and basement membrane of Bowman's capsule

(Fig.2-C).

Histochemical Results:

Liver: Examination of liver sections of control rats stained with periodic acid Schiff's (PAS) technique showed an abundance of glycogen in the form of purple granules and particles in the cytoplasm of the hepatocytes. The nuclei of the hepatocytes gave negative PAS reaction indicating the absence of glycogen (Fig. 3-A). The histochemical examination of D & DE groups showed pericentral depletion of the PAS +ve materials (Fig. 3-B).In diabetic rats treated with wild cherry oil show the distribution of polysaccharides in the liver tissue that appear more or less like control (Fig. 3-C). **Kidney:** Kidneys of N rats showed the presence of polysaccharides in the form of PAS positive materials in the parietal and visceral walls of the Bowman's capsule. Light microscopy of the kidney sections from D&DE rats showed an increase in the thickness of basement membranes and the PAS +ve material in the mesangial cell and matrix of the glomeruli, as compared with the normal control group (Figs. 4- A). DEW & DEC groups showed no effect of these herbs on the kidney tissues (Fig.4-B).

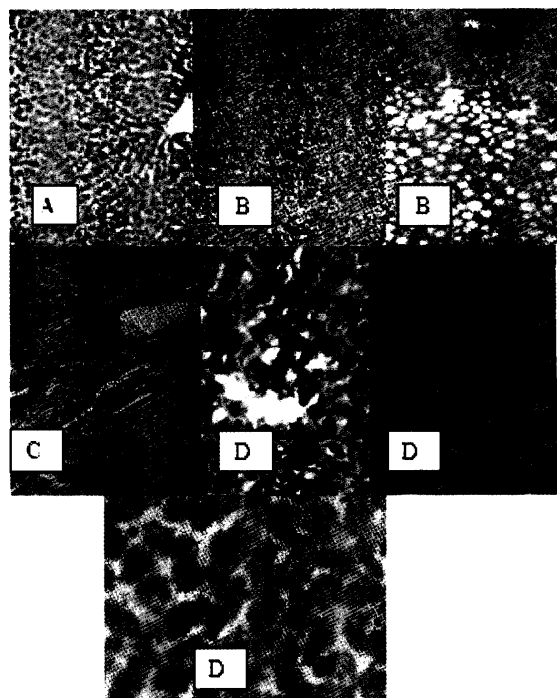


Fig 1: Hepatocytes and hepatic tissue from liver, stained with H&E stain.

(A)-Untreated control rats with normal liver histology (low power).

(B)-Diabeticgroup, showing necrotic and vacuolated cells (medium power)

(C)- DE group showed dilated and congested portal vessels as well as areas of inflammatory cell infiltration.(L.P.) (D)- DEW&DEC group hepatocyte architecture appears more or less like control with some activated cells (cell division in prophase stage) (arrow, H.P.

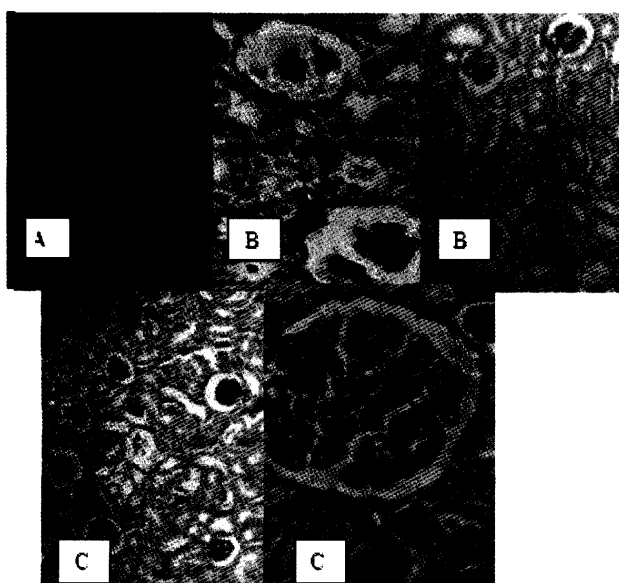


Fig 2: Kidney tissues showed:

- A) kidney of the control rats revealed normal structure of the glomeruli.
 B) kidney of D & DE rats showed atrophy of some and increase in the mesangial cell and matrix of the glomeruli in the others (Medium power).
 C) Kidney of DEW & DEC groups showed no significant differences than D & DE groups (Medium and high power).

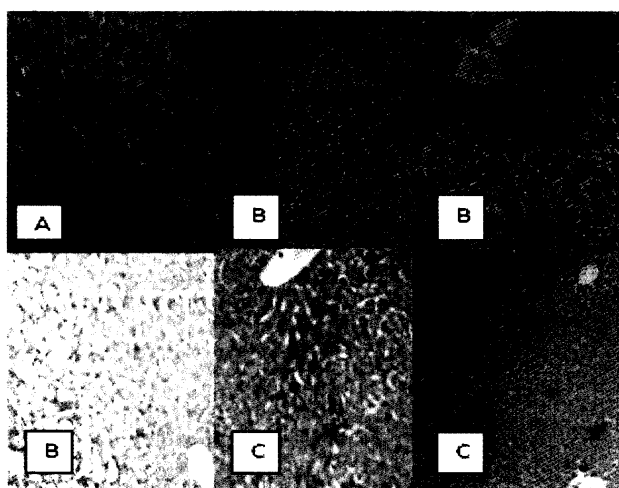


Fig.3: Liver sections (PAS) stain showing:

- A) Normal control appearance of glycogen in the form of purple granules and particles in the cytoplasm of the hepatocytes.
 B) D& DE groups showed pericentral depletion of the PAS +ve material
 C) DEW & DEC groups showed the distribution of polysaccharides in the liver tissue that appear more or less like control.

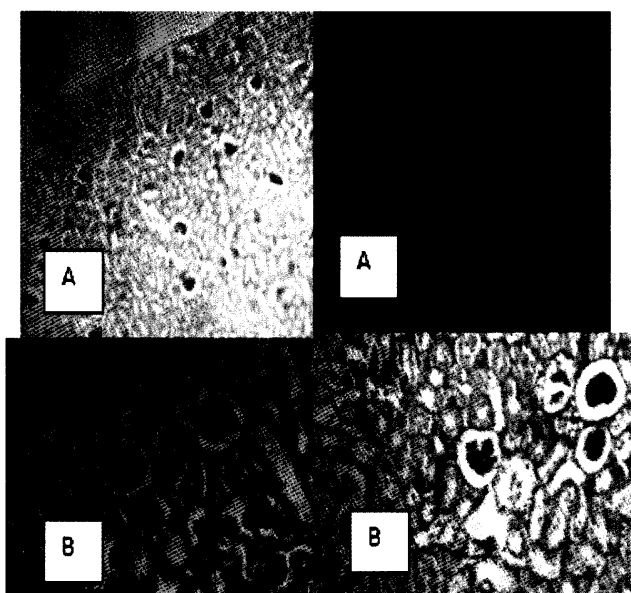


Fig.4: Kidney tissues (PAS) stain:

- A) D & DE groups showed an increase in the thickness of basement membranes and the mesangial cell of the glomeruli (Low and high power).
 B)-Glomerulus and kidney tissue of DE group treated with herbs showed no significance differences, as compared with DE alone (medium power)

Discussion:

Diabetes produces substantial change in the intracellular metabolism in many tissues including liver and kidney (18). The toxicity caused by alloxan is apparently due to injury in β -cell and elevation of local free radicals in these cells after increasing free radicals in other body organs (19). This is supported by elevation of plasma glucose, triglyceride and protein levels which indicated the damage of pancreatic β -cells with the resultant various metabolic disorders (20). Our results are in agreement with (21). From our results it was found that oral administration anthocyanins of wild cherry resulted in significant decrease in glucose level and improved changes in serum lipid & TG. These results are in agreement with Anderson et al. (22) which revealed that the anthocyanins functions as antioxidants, and may be beneficial in the control of glucose intolerance and diabetes, and agree with several studies which reported that these herbs were found to improve the diabetic status, including protection of DNA against oxidative damage and hypoglycemic effects (23). Oral administration of 0.25 gm/kg of *C. cuminum* to diabetic rats resulted in significant reduction in blood glucose, lipid and triglycerides; these findings agree with Dhandapani et al. 2002 (24). In addition, cumin has antioxidant properties (25). These herbs may actually decrease the cellular resistance to insulin, allowing the cells to utilize it more efficiently in metabolizing glucose. It also decreases the absorption of glucose from the small intestine. It reduces the formation of glycogen in the liver.

and increases the uptake and utilization of glucose in the fatty and muscular cells throughout the body. Non-insulin dependent diabetics are able to maintain better blood glucose levels closer to the normal range, while insulin dependent diabetics are often able to reduce their insulin dosage and more easily maintain stable levels of the blood glucose. (26) In the present study, the histopathological and histochemical examinations of the liver and kidney of diabetic rats showed periportal necrosis of the hepatocytes. Dilatation and congestion of the portal vessels, areas of inflammatory cell infiltration and an increase in the mesangial cell and matrix of the glomeruli and hyalinization of arterioles was also observed. These results are in agreement with Buko (27) who reported that the liver of diabetic rats was characterized by hydropic dystrophy and lymphocytic infiltrations. Damages that occur in the liver and kidney of diabetic rat may be due to Oxygen Free Radicals. Meerson (28) stated that Oxygen Free Radicals (OFRs) exert their cytotoxic effect by peroxidation of membrane phospholipids leading to a change in permeability and loss of membrane integrity. Decreased endothelium-dependent relaxation in diabetes is linked to release of OFRs. (29) During diabetes, persistent hyperglycaemia resulted from an increase in glucose oxidation and protein glycosylation. Wautier (30) described that advanced glycation end products on the surface of erythrocytes of diabetic patients enhance their binding to endothelial cells and result in oxidant stress. Cellular defense mechanisms such as antioxidants and antioxidant enzymes offer protection to cells and tissues from oxidative injury (31). The imbalance between OFR production and cellular defense mechanisms could be critical in influencing vascular injury. The increase in the level of OFRs in diabetes could be due to their increased production and/or decreased destruction by non-enzymic and enzymic Catalase (CAT), glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) antioxidants (32). Hyperinsulinaemia increases the activity of the enzyme fatty acyl-CoA oxidase which initiates β -oxidation of fatty acids resulting in increased production of H_2O_2 (33). The present study indicated that cherry oil with anthocyanin and cumin seeds given to the diabetic rats normalized the histological abnormalities caused by diabetes. This improvement may be due to antioxidant effects of these compounds that protect against oxidative stress. As a strategy to counteract the negative effect of oxidative stress, antioxidant-based therapy is promising to minimize the complications associated with oxidative stress in diabetes mellitus. Other observations have shown that many of these complications are diminished upon supplementation with certain dietary antioxidants such as vitamin E, C, and α -lipoic acid (34). The use of other non-nutrient antioxidants such as flavonoids and poly phenols has been reported with the same advantage (35).

References:

1-Grover J.K., Yadav S., Vats V. 200 Medicinal plants of India with antidiabetic potential. *J. Ethnopharmacol.* 81: 81-100 (PubMed).

- 2- Lipinski B. 2001; Pathophysiology of oxidative stress in diabetes mellitus. *J.Diabet.Complications.* 15:203-210.
- 3- Bailly C.J, Aay C: 1998 Traditional plant medicines as treatments for diabetes. *Diabetes Care*; 12:553-504.
- 4-Manisha M., Priyanjali D., Jayant L., Saroj G. and Thomas P.A., 2007; Indian herbs and herbal drugs used for the treatment of diabetes. *J.Clin Biochem Nutr.* 40(3): 163-173.
- 5-Dhandapani S , Subramanian VR , Rajagopal S , Namasivayam N. 2002; Hypolipidemic effect of *Cuminum cuminum* L., on alloxan-induced diabetic rats. *Pharmacol Res.* 46(3):251-5.
- 6-Seymour EM, Singer AAM, Benmink MR, Bolling SF. 2007; Cherry-enriched diets reduce metabolic syndrome and oxidative stress in lean Dahl-SS rats. *Experimental Biology*.
- 7- Lean, M.E.J., M. Noroozi, I. Kelly, J. Burns, D. Talwar, N. Sattar and N. Crozier; 1999 Dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNA. *Diabetes.* 48:176-181.
- 8-Anderson, R.A., C.L. Broadhurst, M.M. Polansky, W.F. Schmidt, A. Khan, V.P. Flanagan, N.W. Schoene, D.J. Graves. 2004; Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *J. Agric. Food Chem.* 52:65-70.
- 9-Jayaprakasam, B., K. Shaiju, L. Vareed, Karl Olson and G. Muraleedaran. 2005; Insulin Secretion by Bioactive Anthocyanins and Anthocyanidins Present in Fruits. *J. Agric. Food Chem.*, 53:28-31.
- 10-Victor M.Pineiro-Carrero, MD and Eric O.Pineiro, MS: 2004, Liver. *PEDIATRICS*, 113, 4, 1097-1106.
- 11-Erythrocim Lactobionate – LV. 2003 Sterile Erythromycin Lactobionate , USP, for I.V. use only, internet.
- 12-Fall JM, Seeram NP, Zhao C, Nair AG, Meyer RA, Raja SN, JM. 2003. Tart cherry anthocyanins suppress inflammation-induced pain behavior in rat. *Behav. rain Res.* 2004; 153(1): 181-8. doi: 10.1016/j.bbr.11.011. ISSN 0166-4328. PMID 15219719.
- 13 - Bown, D. *Encyclopedia of herbs and their uses*. Doreth Kindersley, London, 1995. ISBN 0-7513-020-3. A very well presented and informative book on herbs from a globe. Plenty in it for both the casual reader and the serious one. main quibble is the silly way of having two separate entry plant.
- 14- Manandhar , N.P. *Plants and people of Nepal*. Timber Oregon, 2002 ISBN 0-88192-527-6. Excellent book, covering over 1, 500 species of useful plants together with information on the geography and descriptions of the plants with terse notes on their uses.
- 15- Ohno T, Kitoh J, Yamashita K, Ichikawa Y, Horio E, Terada M, Tanaka S, Namikawa T. 1998; Toxin-induced (D)MI (insulin dependent diabetes mellitus) in the musk shrew. *Life Sci.* 63(6):62-65.
- 16-Szkudelski T, Kudzinski M and Okulicz M. 1998; Alloxan in vivo dose: not only exert deleterious effects on pancreatic B cells. *Physiol -Res*; 47(5):343-6.
- 17-Laurie Barclay, MD: Management of GI Tract Complications Linked With Diabetes Reviewed. *American*

- Family Physician, 2008, June 18.
- 18-Parinandi, N.L., E.D.W. Thompson, H.O.H. Schmid, 1990. Diabetes heart and kidney exhibit increased resistance to lipid peroxidation. *Biochem. Biophys. Acta*; 1047: 63-69.
- 19-Muruganandan, S.S. Gupta, M. Kataria, J. Lal, P.K. Gupta, 2002. Mangiferin protects the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats. *Toxicology*; 15: 165-173.
- 20-Goldstein, D.E., R.R. Little, R.A. Lorenz, J.I. Malone, D. Nathan, C.M. Peterson and D.B. Sacks, 2004. Test of glycemia in diabetes. *Diabetes care*; 27: 1761-1773.
- 21-Anwar, M. and A. Meki, 2003. Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin. *Comparative Biochemistry and physiology part A*; 135: 539-547.
- 22-Anderson, R.A., C.L. Broadhurst, M.M. Polansky, W.F. Schmidt, A. Khan, V.P. Flanagan, N.W. Schoene, D.J. Graves, 2004. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *J. Agric. Food Chem*; 52: 65-70.
- 23-Babu, P.S. and K. Srinivasan. Renal lesions in streptozotocin-induced diabetic rats maintained on onion and capsaicin containing diets. *J. Nutr. Biochem.*, 1999; 10: 477-483.
- 24-Dhandapani S, Subramanian VR, Rajagopal S, Namasivayam N. 2002. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacology Res.*; 46(3): 251-5.
- 25-Martínez-Tomé M, Jiménez AM, Ruggieri S, Frega N, Strabbioli R, Murcia MA., 2001. Antioxidant properties of Mediterranean spices compared with common food additives. *J Food Prot.*; 64(9): 1412-9.
- 26- www.womanfitness.net/articales_ym 2003. Herbal Management of Diabetes.
- 27-Buko, V., O. Lukivskaya, V. Nikitin, Y. Tarasov, L. Zavodnik, A. Borodinsky, B. Gorenshtein, B. Janz, K.J. Gundermann and R. Schumacher. 1996. Hepatic and pancreatic effects of polyenylphosphatidylcholine in rats with alloxan induced diabetes. *Cell. Biochem. Funct*; Jun; 14(2): 131-7.
- 28-Meerson, E.Z., V.E. Kagan, Y.P. Kozlov, L.M. Belkina and Y.V. Arkhipenko, 1982. The role of lipid peroxidation in pathogenesis of ischemic damage and antioxidant protection of the heart. *Basic Res. Cardiol.*, 77: 465-8.
- 29-Tesfamariam, B., 1994. Free radicals in diabetic endothelial cell dysfunction. *Free Rad. Biol. Med.*; 16: 383-91.
- 30-Wautier, J.L., M.P. Wautier and A.M. Schmidt, 1994. Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: a link between surface associated AGEs and diabetic complications. *Proc. Natl. Acad. Sci. USA*; 91: 7742-6.
- 31-Freeman, B.A. and J.D. Crapo, 1982. Biology of disease. Free radicals and tissue injury. *Lab. Invest.*; 47: 412-26.
- 32-Kakkar, R., S.V. Mantha, J. Kalra, and K. Prasad, 1996. Time course study of oxidative stress in aorta and heart of diabetic rats. *Clin Sci.*; 91 (4): 441-8.
- 33-Horie S., H. Ishii and T. Suga, 1981. Changes in peroxisomal fatty acid oxidation in diabetic rat liver. *J. Biochem.*, 90: 1691-6.
- 34-Davie, S.J., B.J. Gould, J.S. Yudkin, 1992. Effect of vitamin C on glycosylation of proteins. *Diabetes*. 1981; 41: 167-173.
- 35- Asgary, S., G.A. Naderi, N. Sarraf Zadeegan, R. Vakili, 2002. The inhibitory effects of pure flavonoids on in vitro protein glycosylation. *Journal of Herbal Pharmacotherapy*; 2: 47-55.