Experimental Study Using Cinnamon Oil for Prevention of Diabetic Nephropathy in Rats

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ABSTRACT:

BACKGROUND:

Diabetes mellitus is the leading cause of chronic renal failure. However, in the early phase of the disease before complications have set in, the glomerular filtration rate is elevated and kidney size increased. The clinical importance of these early aberrations derives from the hyperfilteration (the heightened glomerular capillary) that drives damages the glomerulus, and the enlargement of the kidney (the glomerulus).

OBJECTIVE:

The aim of the present investigations is to examine histologically the effect of cinnamon oil on the kidney tissues of alloxan – induced diabetes rats.

MATERIALS AND METHODS:

Fifty male rats were used and divided into four groups: Group I =12 animal controls, Group II =12 treated with alloxan, Group III = 12 treated with alloxan + cinnamon oil, Group IV = 12 treated with cinnamon oil only.

RESULTS:

Renal tissues (Bowman's capsule, proximal and distal convoluted tubules) of diabetes group: revealed obvious mesangial expansion and basement membrane thickening. While the diabetic treated animals with cinnamon oil ameliorated the increase in the mesangial area in diabetic rats.

CONCLUSION:

Cinnamon oil can be recommended as an support for the prevention of alloxan – induced diabetic complications.

KEY WORDS: renal tissue , diabetes mellitus, cinnamon oil .

INTRODUCTION :

Diabetes mellitus causes narrowing of the small blood vessels throughout the body . It seems that the higher the blood sugar level , the more the small blood vessels narrow . As this happens , the blood vessels carry less blood , and circulation becomes poor and in turn lead to diabetic nephropathy (DN),and eye problems . Diabetes also alters fat metabolism, increasing the risk that cholesterol – laden plague will build up in the large blood vessels [(1)].

The most important criterion of efficiency of diabetes therapy is the effect of treatment on the widespread complications of types I diabetes mellitus. Diabetic nephropathy is one of the most prominent of these complications, and it develops in approximately 25 % - 30 % of all insulin dependent diabetes mellitus patient and is accompanied with a progressive impairment of the kidney function.

The pathogenesis of diabetes nephropathy is characterized by the enlargement of glomerular mesangium due to the accumulation of extra cellular matrix proteins synthesis that leads to glomerulosclerosis and tubulointerstitial fibrosis, basement membrane thickening, increase endothelial cell permeability to albumin , and increase hyper filtration and cause of end – stage renal disease[(2)]. Clinical studies in subjects with type I and type II diabetes clearly link hyperglycemia to vascular complications , including diabetic nephropathy. Hyperglycemia is responsible for the development and progression of diabetic nephropathy through metabolic derangements, including increased oxidative stress, renal polyol formation, release of cytokines such as transforming growth factor – B by glomerular endothelial , epithelial, mesangial, and tubular cells, which results in mesangial hypertrophy, and accumulation of advanced glycation end products, as well as such hemedynamic factors as systemic hypertension and increased intraglomerular pressure[(3)] .

Oxidative stress playing an important role in chronic complications of diabetes. The elevated levels blood glucose in diabetes product oxygen free radicals (ROS) that cause membrane damage due to the peroxidation of membrane lipids and

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protein glycation[4]. Glucose auto – oxidation in the presence of transition metal ions generates oxygen free radicals that make the membrane vulnerable to oxidative damage. Studies have indicated that tissue injury in diabetes may be duo to free radicals[5]. Thus the present study investigated the changes in the antioxidant system in the kidney tissues during diabetes.

Traditionally kidney disease has been considered an irreversible and progressive condition that will eventually lead to renal failure, but recent researches shows that early detection and intensive treatment in people with type II diabetes may actually save kidney function[6] .

Experimentally diabetes induced by chemical agents, such as alloxan , or streptozotocin which damage beta cells of the islets of langerhanse. Several metabolic functional and structural changes in streptozotocin and alloxan–diabetic rats have fundamental similarities to those occurring in human diabetic nephropathy, and this model has been used extensively in diabetes research aiming to elucidate the pathogenesis of diabetic kidney disease. In animal models, complications of various pathogenic factors of DN have been demonstrated involving proliferation of mesangial cells, expansion of the mesangium, thickening of the glomerular basement membrane, glycogen deposits in tubules cells ,and the other major change of DN is renal hypertrophy. This changes often develops in the early phase of diabetes, after alloxan injection. In rats, an evident increase of mesangial matrix and thickening of glomerular basement membrane is seen 3 – 6 months after alloxan injection[7]. In experimental diabetes , mesangeal lesions can be prevented or improved by glycemic control with insulin[3] . Langerhans islet transplantation, and pancreas transplantation

Hamada, lida eIshimura[8]using acarbose associated with insulin in spontaneous diabetic mice, obtained mesangial lesion improvement . and prevented glomerular basement membrane thickening .

There are actually two kinds of diabetes: Type I (insulin–dependent), and Type II (non – insulin dependent). Type II is by fore the more prevalent form of diabetes. People with type II can usually control their blood sugar through weigh loss and diet, sometimes in combination with oral medication that boosts the effect of their own insulin, in addition to exercising and taking supplements,{minerals like anadium,chromium,argentine, lipoic acid,vitamin E}, might also help at least slightly in controlling control blood sugar levels , herbs may play a role in normalized blood sugar levels include fenugreek , gymnema , ginseng , and cinnamon[9].

Cinnamon (Cinnamomum zeylanicum)

Cinnamon of family Lauraceae is considered a useful carminative for the removal of gastrointestinal gas.This herb is an effective digestive aid , and has also been used in folk remedies as a styptic for conditions such as uterine hemorrhage. Recent studies have determined that consuming as little as one–half teaspoon of cinnamon each day may reduce blood sugar, cholesterol, and triglyceride level by as much as 20 % in type II diabetes patient who are not taking insulin[10].Cinnamon trees grow in a number of tropical areas, including parts of India ,China, Madagascar, Brazil, and Caribbean .Typical recommended dosages of ground cinnamon bark are 4 gm daily, cinnamon oil is generally used at a dose of 0.05 – 0.2 gm daily[11].

MATERIALS AND METHODS:

Adult Wister male rats weighing 200 – 300 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 ad labium ,they were also maintained under slandered conditions of humidity, temperature and 12 hr light / cycle. Rats were injected intraperitoneally to starved animal with a freshly prepared solution over 10 minutes of alloxan monohydrate in normal saline in a single dose of 100 mg / kg body weight[12]. Because alloxan is capably 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 hyperglycemia[13].Animals were randomly assigned into 4 groups as shown in the following table I :

Table I : Showing animal groups

Groups	Sub - groups	Number of animal	Notes
Control	C	12	Given wateronly
Experimental	D	12	Given alloxan
	DC	12	Given alloxan +cinnamon Oil*
	C	12	Given alloxan oil only

N = Control group.
D = Diabetic group.
DC = Diabetic animals treated with cinnamon oil.
C = Cinnamon oil, given orally as a dose of 0.2 ml / day (11) throughout the time of study.

Each group was subdivided into 4 groups corresponding to sacrifice at, 14, 28, 56, 84 days after diabetes induction. Kidney was taken from anaesthetized animal using Nembutal solution (0.06 ml / gm body weight) (S.SN. A. LaBallasler ,33501 Libourne cedex France), body weight of animals (before and after treatment),organ weight (kidney) of each animals was then calculated as follows :

$$\text{Re}\,lativeoragan = \frac{Absoluteaveragewight\,(gm)}{Bodyweight\,ofratonscarifiedday\,(gm)}$$

Blood glucose level was determined for biochemical studies.

Quantitative morphological studies of kidney lesions:

At the end of the study period, kidneys were taken from the scarified animals, the right kidney were weighted , and the left one was fixed in 10 % buffered formalin for 24 hrs, washed with tap water , dehydration using 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 u thick . After deparartfinization formalin – fixed sections of the left kidney were stained using hematoxylin and eosin, periodic acid – shiff (PAS) stains[14], mounting with DPX. Fifty glomeruli and the related tubules from each kidney were examined by light microscope. Quantitative morphologic measurements of glomerular mesangium and Bowman's capsule were made by using an eye piece micrometer. The results are expressed as mean ± standard. For statistical comparisons among the experimental groups, F – test was used to examine difference between mean values of the groups , and was used to evaluate mesangial changes over the study period , at P – value less than 0.05 was considered to be significant .

RESULTS:

Body weight: The body weight of (D) rats was statistically lower than normal (N). The (DC) rats showed semi – similar weight to N rats, as shown in (Table II).

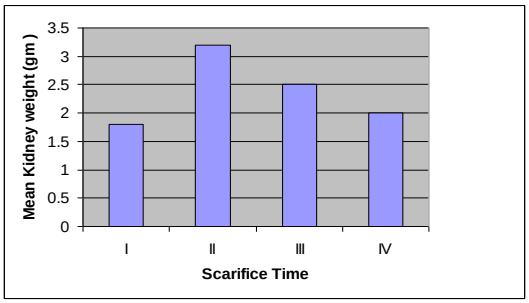
Table II: Showed the body weight in the different groups throughout the Study period.

Sub - groups	2nd weeks	4th weeks	8th weeks	12 weeks
N	200-0.8*	250-5.0*	230-3.0*	200-0.5*
D	120-4.5*	150-3.6*	150-4.1*	100-3.0*
DC	190-1.5*	220-2.8*	230-3.8*	210-2.5*
C	210-3.0*	250-2.5*	250-1.5*	230-2.5*

- Significant at P < 0.05 .
- Data are expressed as means ± standard deviations (SD) .

Kidney weight

During the study period kidney weight increased significantly in normal group rats (P < 0.05,Fig.I). Diabetic group rats showed a significantly greater increase in kidney weight during the study period when compared to control group (P <0.05) . In diabetic treated with cinnamon group showed significantly lower kidney weight compared to diabetic group rats (P < 0.05) .



I : Control group
II : Given alloxan only
III : Given alloxan + cinnamon
IV: Given cinnamon oil only

Fig I: Effect of diabetes and cinnamon oil on kidney weight .

Blood glucose :A significant increase(p< 0.05)in blood glucose (Table III)[Estimated by the glucose oxidase method] was evident in diabetic animals when compared to non- diabetic rats (N) and cinnamon- diabetic treated animals.

Table III : Changes in blood glucose during the study period in N, D, DC, C groups

Sub- groups	NO.	Glucose(mg/dl) 2nd weeks	Glucose(mg/dl) 4th weeks	Glucose(mg/dl) 8th weeks	Glucose(mg/dl) 12th weeks
N	12	100-1.6*	110-3.0*	120-1.1*	120-2.0*
D	12	300-3.5*	380-3.0*	280-2.0*	280-3.4*
DC	12	150-2.5*	130-3.6*	120-2.5*	120-2.5*
C	12	140-2.0*	130-2.5*	130-4.0*	100-1.5*

*Values are mean ±SEM, P < 0.05 compared with non – diabetic groups.

Renal lesions: A gradual mesangial and enlargement (ME) was observed in the diabetic groups (D) (Fig.2) from the 2nd weeks to the end of the experiment after diabetes induction(TableIV).The DC rats(Fig.3) showed similar ME to that of non – diabetic rats (N) for the same period (Fig .4) .

Table IV: Mesangial enlargement – showed the differences in diameter of the statistical test of experimental groups, and sacrifice time (Low power,u m) .

Groups	NO.	Sacrifice Time			
		2nd weeks	4th weeks	8th weeks	12th weeks
N	12	12.37 – 1.5*	11.5 -2.0*	12.5 -3.5*	12.5 -3.4*
D	12	18.52-3.5*	17.9-3.5 *	19.68-3.0*	18.2 -3.5*
DC	12	11.6-83.0*	13.32 -2.5	12.48-4.5*	13.5 -5.0*
C	12	10.5-2.2*	11.3 -4.4*	10.7 -3.2*	12.5 -3.5*

*Significant at P < 0.05 .Data are expressed as means ± standard deviations (SD) .

Table V: Showing differences in diameter (µ m) of the Bowman's capsule throughout the investigation .

Groups	NO.	Sacrifice Time				
		2nd weeks	4th weeks	8th weeks	12th weeks	4th month Wide length
N	12	12.06 -3.5*	11.5 -2.5*	12.3 -3.0*	12.5 -2.3*	
D	12	18.5 -2.5*	17.5 -3.5*	18.9 -3.5*	18.0 -3.2*	
DC	12	15.7 -5.0*	15.7 -4.0*	16.9 -2.5*	13.5 -3.4*	
C	12	15.6 -3.5*	13.3 -3.5*	15.8 -2.5*	11.8 -3.5*	

Significant at P < 0.05 .Data are expressed as means ± standard deviations (SD).

While table VI shows the medians of Armanni – Ebstein lesions, consisting of renal tubule cell vacuolization and distension due to glycogen accumulation (Fig.5). While diabetic groups treated with cinnamon oil revealed semi – similar to N tubules with less vacillation of the tubules (Fig .6) .

Table VI: Revealing differences in the diameter (µm) of Armani –Ebstein lesions in tubules throughout the investigation .

Groups	NO.	Sacrifice Time			
		2nd weeks	4th weeks	8th weeks	12th weeks
N	12	0.71-3.2*	0.8 – 3.2*	0.71 – 3.0*	0.62 -3.2*
D	12	18.6 -3.5*	19.2 – 4.0*	18.5 – 2.5*	18.2 -3.4*
DC	12	15.3- 2.3*	10.5 – 3.5*	12.5 – 2.5*	11.2 – 2.5*
C	12	1. 2- 2.0*	0.99 - 3.0*	1.5 - 2.5*	0.87- 3.0*

*Significant at P < 0.05 .Data are expressed as means ± standard deviations (SD) .

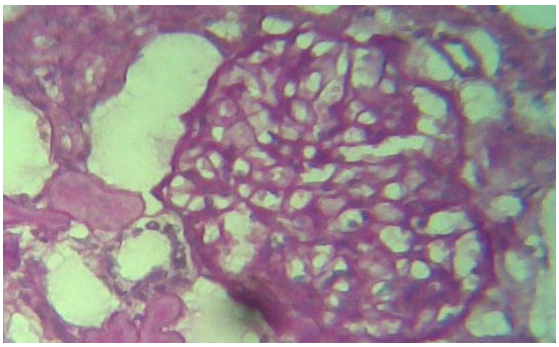


Fig 2: Glomerulus from an untreated diabetic rat at 2nd weeks of age , showing glomerular hypertrophy and increase in mesangial matrix expansion (PAS) . X 40 .

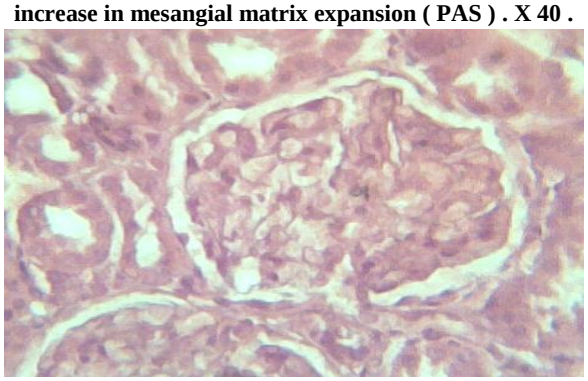


Fig 3 : Cinnamon oil , depicting partial reversal of mesangial matrix expansion (H & E) . X 40 .

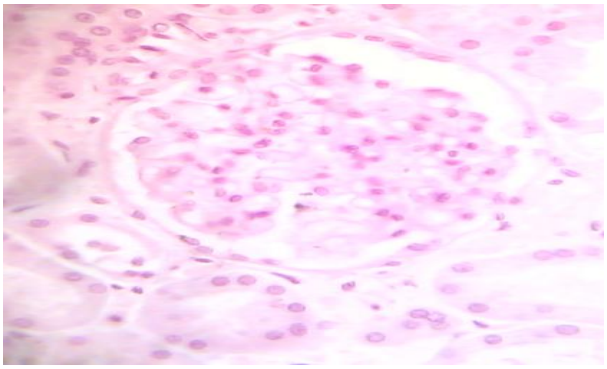


Fig 4: Histological characteristics in non – diabetic rats , shows normal appearance in control rats (tissue) (H& E) . X 40 .

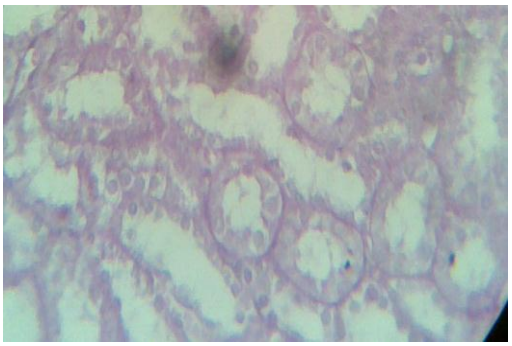


Fig 5: Tubules of D rats sacrificed at 2nd weeks after diabetes induction , showing severe glycogenic vacuolization (clear cells) (H & E) . X 40 .

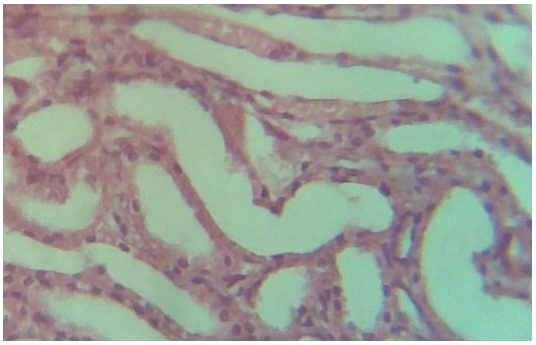


Fig 6: Tubules of DC rats showing semi-similar to N tubules with less vacillation of the tubules (H & E) . X 40 .

DISCUSSION:

The diabetic pathological complications , in both types (I and II), are mostly due to excessive elevated production of reactive oxygen species (free radicals) in diabetes , and the role of these toxic species on kidney tissues and the antioxidants defense system have been studied .treatment with cinnamon -oil was followed to measure its effect on the antioxidant potential alloxan – induced diabetic nephropathy .

In this context, along 3 months of follow – up we observed a significant decrease in blood glucose levels, body and kidney weight, and glomerular histological changes in DC group when compared with D group. The results showed increase in blood glucose levels in alloxan – induced diabetic rats; this coincides with previous investigation in this respect which proved that alloxan is a specific β – cytotoxic agent[15]. The elevated of blood glucose levels that, with auto – oxidation, generate free radicals (5). Alloxan also has been shown to produce oxygen free radicals[16].In alloxan diabetic group we observed a significant increase in body and kidney weight when compared with control (N)group, while cinnamon treated animals revealed a significant hyperglycemia ($P< 0.05$) as shown in table III when administrated orally to diabetic group, and improved body weight parallel to previous studies[17]. This reduction may be as an increased gluconeogenesis in the liver , which show an increased turnover rate , this involves a negative ATP balance[18] or it may be suggested that treated with cinnamon leads to increase energy storage in subcutaneous adipose site [19], because diabetes is characterized by decreased insulin secretion[20], and sensitivity in liver, adipose tissue, and skeletal muscle . The increment of kidney weight after onset of diabetes on day 14 agree with tayfun et al , 2000 [21] who reported that kidney weight increased significantly 7 days after alloxan administration .

Mesangial hypertrophy has been observed in diabetic rats by light and electron microscopy between 6- 12 months after diabetes induction[22]. Moreover, previous studies on kidney functions in alloxan diabetic rats[23] revealed early glomerulopathy among these rats as evidenced by a significant increase in renal tubular dysfunction in the diabetic condition of the 5 weeks follow up periods , namely by the end of the fourth and fifth weeks from the onset of the disease. Such finding

runs parallel with our study with the detection of glomerular dysfunction the early stage of diabetic nephropathy, by means of significant increment of glomerular diameters in the early stages of diabetes. In addition, on day 14 of diabetes revealed expansion of mesangium and thickening of the glomerular basement membrane. This suggests the injury begins from the early stages.

In this connection it is of interest to add that the relation ship between diabetic duration and the development of renal damage. Five different stages have been suggested, the first stage is characterized by a hyper function, i.e. , an increase the glomerular filtration rate. In the second stage a glomerular damage develops without clinical symptoms. In the third stage, an incipient nephropathy with the microalbuminuria is observed . In the last two stages, the proteinuria increases and the kidney damage advances to an irreversible state with a progressive kidney insufficiency. Thus , it seems of great necessity that early and follow up elevation of the glomerular as well as the tubular function in diabetes is of prim importance to enforce necessary medical care to avoid further complications of the diabetic nephropathic conditions among diabetic patients .

The mesangium is an intracapillary network of mesangial cells and matrix that is contiguous with the circulation through a layer of endothelial cells with fenestrate. The advanced glycation end products sieved through the mesangium and mesangial receptors for these products in rats play an important role in mesangial matrix increase[24].

Many other pathogenic factors are involved in mesangial matrix increase. Sharma et al. 1996, using antibody to TGF – B, reported a prevention of glomerular hypertrophy and partial normalization of matrix synthesis[25]. In fact, enhanced renal expression of TGF – B protein and mRNA has been reported in a range of glomerular diseases. According to these authors, hyperglycemia leads to hyper filtration, and mesangial stretch serves as a single for increased production of TGF – B by mesangial cells, this cytokine being the causative factor of increased mesangial matrix synthesis. In addition to matrix synthesis increase, diabetic rats showed a decrease of metalloproteinase activity and smaller degradation rate of extra cellular matrix compounds [26].

In uriniferous tubules apparent structural alteration were observed in proximal straight tubules epithelium , vasodilatation observed in the diabetic groups , hyaline material (homogenous material) were observed in the glomerular by using PAS stain .

In diabetes, many mechanisms, such as cellular, homodynamic, and increase of formation of advanced glycation end products[27], caused by hyperglycemia account for mesangial increase. Then, the use of drugs for glycemia control plays a major role in the prevention of mesangial expansion, and as a consequence, renal dysfunction.In this research, cinnamon – oil fed diabetic rats for 3 months (12 weeks) attenuated the mesangial matrix accumulation that had been established from the 2nd weeks of age. These results are agree with the diabetic controlled shown by the alloxan – diabetic rats treated with insulin (3), they presented lower body weight and prevent mesangial increase (expansion). Besides, the glomerulus contained less PAS – positive matrix material and the capillary loops were less open following cinnamon oil. Mesangial expansion and tubular was further quantitated by a morph metrical analysis. Administration of cinnamon oil significantly ameliorated the increase in the relative mesangial area in diabetic rats (Fig. IV).

The possible mechanism by which cinnamon brings about its anti hyperglycemic action may be stimulation of surviving cells to release more insulin [28]. Besides we have shown that cinnamon also function as potent antioxidants, which would lead to additional health benefits of this substance. Duhuley, 1999[29] showed that cinnamon displays antioxidant activity in rats fed a high - fat diet. Other investigators , showed that , cinnamon reduced serum glucose, triglyceride , total cholesterol levels in people with type II diabetes , so cinnamon may play beneficial for the remainder of the population to prevent and control glucose and blood lipid levels [30] .

In our review of literature , we didn't find any research about the effect of cinnamon on the kidney tissues (Medline 1965 – 2008) .

CONCLUSION:

These observations show that the cinnamon possesses antioxidant activity, which may exert a beneficial action against pathogenic alterations caused by the presence of superoxide and hydroxyl radicals in alloxan – induced diabetes. This action could involve mechanisms related to scavenging activity.

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