The role of Cinnamon – oil on renal tissue of hamster following Alloxan – induced diabetes . Histochemical approach

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SUMMARY:

Diabetes mellitus is a heterogeneous group of disorders characterized by abnormalities in carbohydrate ,protein , and lipid metabolism ,which is due either to insulin production or action . Diabetes is a leading cause of blindness , kidney disorders , disease of small blood vessels ……and various other malfunctions. So the

AIM:

of this study is to investigate the effect of cinnamon oil as a natural source of a wide scale of antioxidants on prevention and treatment of oxidative stress in alloxan – induced diabetic animals , through biochemical and histochemical studies.

MATERIALS AND METHODS :

Thirty – five hamsters were employed in this study . They were divided into 4 groups . 1st group : 5 = animal control , 2nd group :10 = treated with alloxan , 3 rd group , 10 = treated with cinnamon oil and 4 th group , 10 = treated with alloxan and cinnamon .

RESULTS :

Renal tissues (Bowman's capsule, proximal and distal convoluted tubules) of alloxan – treated group revealed obvious increase in acid (ACP) and alkaline (ALP) phosphatase activity . While the 4 th group showed clear decrease in the activity of these enzymes as compared with group I & group III .

CONCLUSION :

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

KEY WORDS : Acid phosphates, Alkaline phosphatase , Cinnamon , Kidney's tissues.

INTRODUCTION :

Diabetes mellitus is a metabolic syndrome characterized by an inappropriate elevation of blood glucose level , and it is associated with many complications [1] .

Enzymes are a part of every metabolic processes in the body . When certain enzymes are in excess in the blood stream and tissues , they can be used for diagnosing specific disease . Enzymes not only play a role in digestion , but also during disease and other metabolic processes [2] .

Acid phosphatase (ACP) (EC 3.1.3.2.) is a family of enzymes that can identified as a group of orthophosphoric monoesterases of the endosomal / lysosomal compartment wit h an acidic PH (5.0) optimum [3,4] . ACP is widely used as a biochemical marker for lysosomes , it has played in the discovery and histochemical visualization of lysosomes [5] neither in vivo substrates nor its functional role . lysosomal storage was found in podocyte and tubular epithelial cells of kidney, in kupffer cells ,macrophages , spleen and in subset of microglia , ependymal cells , and astrglia within the central nervous system [6] . Autoimmune diseases such as diabetes mellitus are associated with highly activities of ACP serum [7] . Sensitive inhibitor of ACP is L – tartrate [8,9] .

Alkaline phosphatase (ALP) (EC 3.1.3.1) is a membrane – bound enzyme that present in most , if not all , organisms . They catalyze the hydrolysis of phosphomonoesters with release of inorganic phosphate and alcohols [10] . ALP activity is present at higher level in kidney , bone , liver and ovary , and at lower levels in many other tissues [11] , and it has been proposed as a marker of preneoplastic changes [12] .

In diabetes mellitus , major contributions were in the field of hypoglycemic action of various plant products , one of them is cinnamon **. Cinnamon** , scientific name= Cinnamon zeylanicum , family name = Lauraceae , Arabic name = Qirfa , local name = Darsin [13 , 14] . This herb traditionally used in treatment of anorexia , blood sugar control , digestive disorders , fever , antifungal , antibacterial , back pain , larvicidal [15] . Few researches elicited that cinnamon oil potentates the

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activity of insulin [16, 17], and improve insulin and glucose metabolism[18, 19]. Therefore the **aim** of the present study is to ameliorate (or prevent) the complications caused by alloxan by using cinnamon – oil .

MATERIALS AND METHODS :

Thirty-five Syrian golden male hamster, were used in this study . They were isolated in a relatively controlled environment at a temperature of about 25 ^{o} C in the "animal Breeding Center " / College of Medicine / University of Baghdad . Animals were given food ***ad libitum*** and free access to water . Animals were divided into 4 groups (Table I) .

Table I : Showing animal groups that were involved in this investigation

Group	Number	Notes
I	5	animal Control *
II	10	Treated with Alloxan**
III	10	Treated with Alloxan +Cinnamon***
III	10	Treated with Cinnamon oil

* Given water only .
** Alloxan was given to a starved animal in a single dose of 100 mg / kg body weight , intraperitoneally over 10 minutes [20,21] .
*** Cinnamon oil ,given orally as a dose of 0.2 ml / day [22] through out the time of study .

A small piece of kidney and another from liver or spleen (used as a positive control for the enzymes) of anaesthetized animals (using Nembutal 0.06 ml / gm body weight) were removed , fixed in 2 % paraformaldehyde[23] . 2 cc of blood was obtained through intracephalic puncher & send to biochemical test . The lead method after Gomori 1952 [24] was used for cytochemical localization of acid & alkaline phosphatase . Cryostat – cut sections were incubated in Colombia jars at 37 ^{o} C (using water bath) , 60 minutes for ACP ; 45 minutes for ALP , in a freshly prepared incubating medium containing :

1. **For ACP** : 20 mg of lead nitrate , 0.05 M acetate buffer PH (5.0) , 32 mg of B – glycerophsphate (as substrate) .
2. **For ALP** : 10 ml of 3 % B – glycerophosphate , 10 ml of 2 % sodium dimethyl barbiturate , 1 ml of 5 % magnesium sulphide .

Twelve sections from each animal (sub-grouped to 4 sets) and two of the liver were involved in above treatment (Table 2) .

Table 2 : Exhibits the kidney's tissue and liver sets of sections used in this study

organ	No. of sets	No. of sections	Used for
Kidney's tissue	1	3	ALP demonstrated
Kidney's tissue	2	3	ACP demonstrated
Kidney's tissue	3	2	Omission of substrate (negative control)
Kidney's tissue	4	2	Specific inhibitor (negative control)
liver	5	2	Positive control for each enzyme

Black precipitate indicates the site of enzyme .
The intensity of the final reaction product of these enzymes was subjectively assessed by double – blind assessment of its final reaction products . Grades were selected in range of 0 – 5 (n = no , 1 = very weak , 2 = weak , 3= moderate , 4 = intense and 5 = very intense activities) . Finally results were statistically analyzed using " T– test " and " F– test " , at P – value less than 0.05 was considered to be significant [25].

RESULTS :

I : Biochemical results :

A significant increase ($p < o.o5$) in blood glucose (Table 4) and obvious elevation in ACP & ALP phosphatase activities level

(Table 5) were found in alloxan - induced diabetic hamster as compared with the appropriate control group and cinnamon treated animals . While a remarkable decrease in the concentration of these parameters , below the diabetic group , was observed in the case of animals treated with cinnamon oil (Table 4 & 5) (p < o.o5) .

Table 4 : Revealed blood sugar levels in both control and experimental groups

Groups	Fasting blood sugar level (mg / dI)
I	90 ± 2.4*
II	190 ± 3.6 *
III	120 ± 2.8 *
IV	100 ± 2.0 *

Data are mean ± standard deviation.
- significant at P <0.05 .

Table 5 : Showing ACP & ALP phosphatase levels in serum of both control and experimental animal groups

Groups	ACP (UIK)	ALP (UIK)
I	12	33 ± 2.o *
II	20	50 ± 3.o*
III	13	36 ± 2.8 *
IV	10	32 ± 2.5*

Data are mean ± standard deviation.
* significant at P < 0.05 .

II : Histochemical results :
After incubation, brown - black precipitation indicate the site of ACP enzyme, while black precipitation revealed to ALP. Renal tissues revealed strong significant (P < 0.05) activities for ACP & ALP in alloxan – induced diabetic animals localized in the brush border of proximal convoluted tubules , and filled the cytoplasm that lies adjacent to the lumen in distal convoluted tubules (Fig. 2) (Table 6) . It was slight in activity in renal glomeruli , as compared with control group (Fig. 1) .

Table 6 : Showing subjective double – blind assessment of ACP & ALP final reaction in experimental groups

Groups	ACP	ALP
I	2	2
II	5	5
III	2	2
IV	3	3
Liver	5	5

Liver was employed to be used in the evaluation of enzymes final reaction product.
Administration of cinnamon – oil significantly (P < o.o5) reduce ACP, ALP activities (Fig. 3) and revealed features of normal tissues as compared with alloxan – induced tissues.

DISCUSSION :

In both type I and type II diabetes mellitus the late diabetic pathological complications are mostly due to excessive elevation production of reaction oxygen species [26]. Therefore , an additional natural antioxidants may be of a great importance in such cases[27]. Various natural products have long been used in traditional medical system for treating diabetes [28] ,therefore , it might be assumed that these products could play very important role at least in the case of non – insulin – dependent diabetes mellitus (type II).

In this study , the significant increase in serum glucose levels in diabetic mice coincides with previous investigations in this respect which proved that alloxan is a specific β- cytotoxic agent [29].

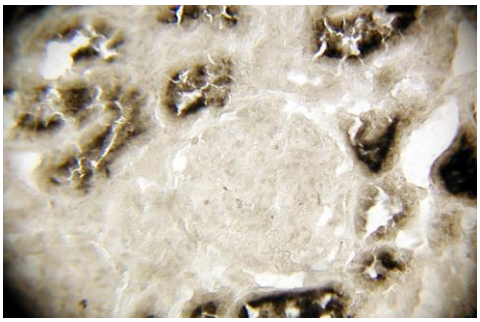
Augmentation of enzymes activity in renal tissues (proximal and distal convoluted tubules) elicited significantly ($P < 0.05$) (Table 6) in diabetic animals . This finding was similar to previous studies [7].

Lysosomal enzymes are known to be involved in cell death and tissue damage of various diseases[30, 31] . The increase in the activity of this lysosomal enzyme , after treated with alloxan suggests increase tissue catabolism and cellular autophagy , which are possible sequence leading to damage of cells[32,33]. Besides , the slight activity in the glomeruli in diabetic animals due to the slight glomerular injury in early stage , and this agree with Maeda 2003 [34] .

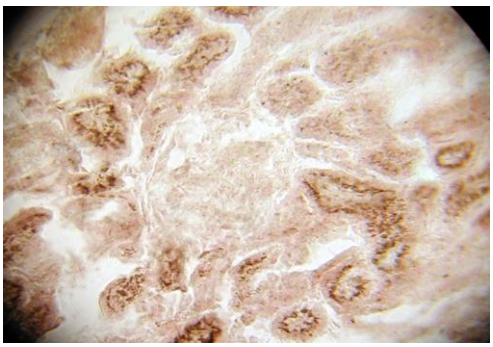
These findings associated with the total serum ACP &ALP activities which revealed significantly higher in autoimmune diseases when compared with the appropriate control groups . This agree with other worker[35]. Blood serum level of ACP & ALP activity is measured in diagnosing specific diseases to evaluate the presence of diseases and metabolic processes such as diabetic mellitus , so the evaluation of these enzymes in the serum could be due to the leakage of them from cells that were killed or injured by autoimmune processes and (or) by abnormal cell activation [6] .

On the contrary , cinnamon - oil fed diabetic mice revealed significant ($P < 0.05$) decrease in ACP & ALP activities in proximal and distal convoluted tubules and trivial activity in glomeruli , with significant decrease of serum activities as compared with these treated with alloxan alone . these result coincides with previous investigation on herbs elsewhere [36,37] .

To the best of our knowledge , I did not find any research (in my review of literature from 1965 – 2006) about the mechanism of this protective action , but most probable phenomena seems to be that alloxan may decrease or may inactivate the average number of free sulfhydryl groups (SH – groups) that are necessary for insulin synthesis in β – cells [38,39] leading to reduction of insulin production , so treated with this herbs may increase the free SH – groups in blood stream which in turn may react or combined directly with alloxan [40] forming inactive compound (dialuric acid) which is a non – diabetogenic [38]. these findings correct the serum glucose level and therefore correct the diabetogenic complications (dysfunction of renal tissue) , our findings showed that cinnamon exert antioxidant and antihypoglycemic effects and consequently may alleviate renal damage caused by alloxan - induced diabetes . further studies is ultrastructural investigation to have more satisfactory results .

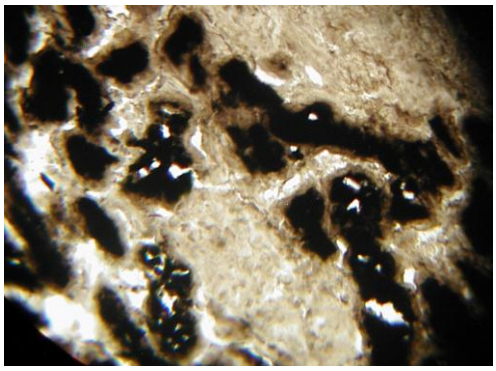


ALP (x 400)



ACP (x 400)

Figure. I :Kidney's tissue: renal tubules (one arrow) , renal glomeruli (two arrows) shows ACP& ALP activities in control hamster



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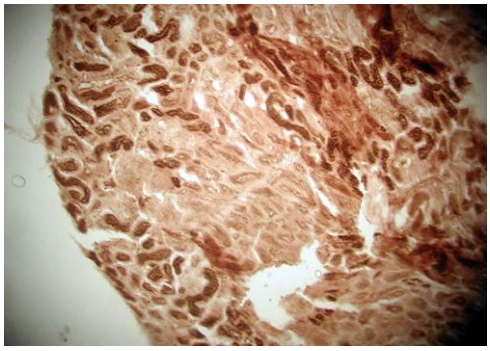
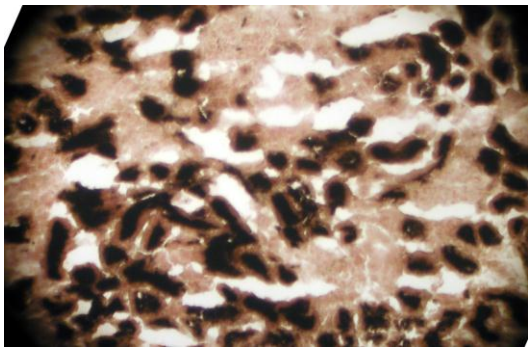
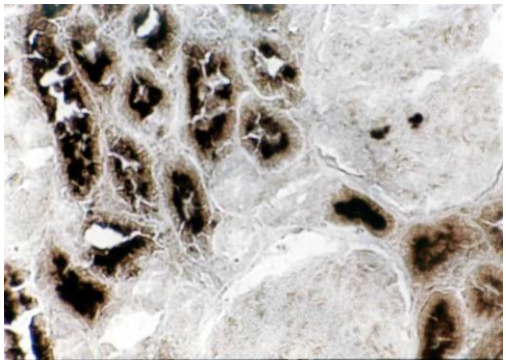


ACP (x 200)

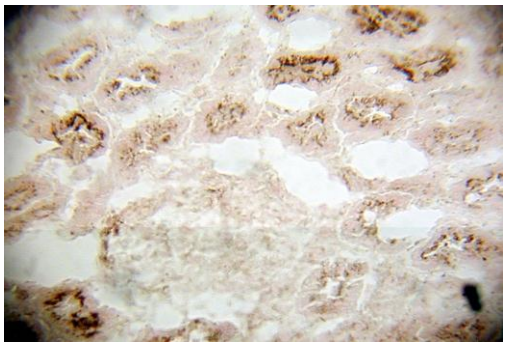


ACP (x 400)

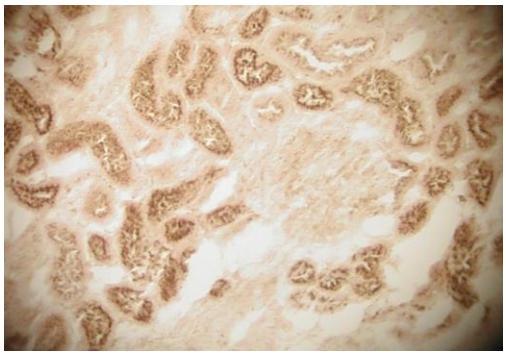
Fig. 2 : Revealed intense activities of ACP & ALP in renal tubules (one arrow), and weak activity in renal glomeruli (two arrows) of alloxan – treated Hamster.



ALP (x 400)



ACP (x 400)



ACP (x 200)

Figure 3 : ACP & ALP activities in of alloxan – cinnamon – treated animal , shows significant decrease in their activities in proximal and distal convoluted tubules (one arrow) and trivial activity in glomeruli (two arrows)

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