The Biochemical and Histological Effect of Cadmium Chloride on the Pancreas of Adult Albino Rats and the Possible Protective Effect of Nigella Sativa Seeds

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ABSTRACT

Aim: The current study evaluates the important role of Nigella Sativa against cadmium (Cd) induced toxic changes in pancreas of rats.

Materials and Methods: Eighteen adult Wistar albino rats were divided into three equal groups. Control group, cadmium treated group that received 5 mg CdCl$_2$/kg BW/day dissolved in water orally for three months, and cadmium with Nigella sativa (NS) group received a mixture of 1g/kg/day of aqueous suspension of Nigella sativa seeds powder and 5 mg CdCl$_2$/kg BW/day dissolved in water for three consecutive months. Hormonal assay of insulin was done. Pancreatic tissues were prepared for both Hematoxylin and Eosin and toluidine blue. Electron microscopic study of pancreatic tissues was also done.

Results: The present study showed that the administration of CdCl$_2$ induced evident decrease in insulin serum level. The biochemical results were confirmed by histological investigations where degenerative changes of pancreatic acini with loss of normal lobular architecture, distorted pancreatic acini, degenerated and widely separated cells in the islets of Langerhans, blood vessels congestion and aggregation of perivascular cellular infiltrates were detected in pancreatic tissues stained with H&E. Toluidine blue-stained sections showed few deeply stained apical zymogen granules in some acini, whereas other acini showed absence of these granules. Administration of NS with CdCl$_2$ showed a significant improvement in the measured serum insulin level and histological pictures.

Conclusion: CdCl$_2$ induced several toxic changes in the exocrine and endocrine parts of pancreas of rats. NS could protect the pancreas against these alterations.

Key Words: Cadmium chloride, histological changes, Nigella sativa, oxidative stress and toxicity, pancreas.

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INTRODUCTION

Cadmium (Cd) is known to be one of the harmful natural and modern pollutants. Its industrial applications were developed based on its unique characteristics[1]. It accumulates in several organs for example, liver, testis, kidney, lung, brain, bone and blood system and influence serious injury to these organs[2], [3].

Cd exhausts protein-bound sulfhydryl groups and glutathione leading to increase formation of reactive oxygen species (ROS)[3]. Oxidative damage to cellular organelles through production of (ROS) was also one of the harmful impacts of cadmium. Cellular biomolecules when reacting with these ROS resulting into peroxidation of lipid, injury of protein membrane, changed antioxidant system, DNA injury, changed gene expression and apoptosis[4-5], [6].

The black seed of Nigella Sativa (NS) was commonly used for the management of asthma in Asia, the Far East, the Middle East and northern Africa[6]. The seeds contain essential and fixed oils, saponin, proteins and alkaloids. Their action was proved to be due to thymoquinone, the main substance of the essential oil, but it is also present in the fixed oil[7].

Also, it was utilised as a natural substance in treatment of rheumatism, hypertension, diabetes, inflammation, cough, headache, eczema, fever and influenza[7,8,9]. Therefore, the goal of this study to test the possible beneficial effect of NS seeds on Cd treated pancreas by histological and biochemical assessment.

AIM OF THE STUDY

To study the histological and biochemical changes of CdCl$_2$ on the pancreas of adult albino rat and the hypothesized protective effect of NS seeds.
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MATERIALS AND METHODS

Cadmium chloride (CdCl₂) was purchased from Standard lab, dissolved in water as a vehicle and Nigella Sativa seeds were obtained from a natural herb market in Alexandria City, Egypt. One gram of Nigella Sativa seeds (black cumin) Fig. 1 was grinded by a blender and mixed with 100 ml of distilled water. This was repeated several times at room temperature until complete grinding and mixing to obtain a stock solution of an aqueous suspension[10].

EXPERIMENTAL ANIMALS

This study was carried out on eighteen adult healthy male Wistar albino rats, each of average weight ranging from 200-250 g or 6-8 weeks of age. The rats were obtained from the animal house of department of Anatomy and Embryology, Faculty of Medicine, Al-Mowasat. The animals were maintained under standard laboratory conditions of temperature and humidity and 12 hours light/dark cycle. The present study was approved by the Ethical guidelines of Alexandria University on laboratory animals and the National Institute for the Care and Use of Laboratory Animals. Further the Alexandria faculty of medicine ethical committee approval was obtained.

In the experiment, the animals were randomly divided into three equal groups:

Control group: included six adult rats that were given standard balanced diet for three consecutive months.

Cadmium treated group: included six adult rats that received 5 mg CdCl₂/kg BW/day[11] dissolved in water orally for three consecutive months.

Cadmium with Nigella sativa group: included six adult rats that received a mixture of 1g/kg/day of aqueous suspension of Nigella sativa seeds powder (Nigella Sativa seeds were grinded and dissolved in water) and 5 mg CdCl₂/kg BW/day dissolved in water for three consecutive months.

At the end of the experiment (three consecutive months), the rats were sacrificed by inhalation of diethyl ether. Blood samples were collected for the extraction of serum (centrifugation for 10 min at 4,000 x g) for hormonal assay of insulin. Serum insulin concentration was measured using mouse insulin ELISA kit (F5618-B, SANJIA, China).

For light microscope:

Pancreatic tissues from the tail were extracted, sliced into small sections, fixed in 10% formalin for 24 hours and were embedded in paraffin. Samples were cut into 5μm thickness sections then stained with Hematoxylin and Eosin (H&E)[12]. Semithin sections 1 μm were cut using LKB ultramicrotome and stained with toluidine blue[12].

For transmission Electron Microscopy:

Small pieces (1 mm) of pancreatic tissues were cut and fixed in a 3% glutaraldehyde phosphate buffer and post fixed in a 2% osmium tetroxide phosphate buffer then embedded in araldite resin. Ultrathin sections were cut and stained with uranyl acetate and lead citrate. Tissue sections were evaluated using a JEOL transmission electron microscope[12] at Faculty of science, Alexandria university.

Statistical Analysis:

Results were interpreted as mean ± standard deviation (M±SD). Statistical analyses were performed with IBM SPSS statistics, version 23.0 (IBM Inc). All experimental results were analyzed utilizing one-way variance analysis (ANOVA) with Dunnett’s post hoc test. Values of \( P \leq 0.05 \) were significantly different.

RESULTS

No death occurred either in the control or in the test groups during the whole period of the experiment.

Hormonal Assays

The results of this study have been appropriately summarized, tabulated and subjected to statistical analysis before completion and graphic illustration. ANOVA test was used in this study to compare between means of different groups.

The obtained results in Table 1 showed a marked significant decrease (\( P \leq 0.05 \)) in serum insulin concentration in the Cd treated group compared with control group. Cd treated group with Nigella Sativa nearly returned these values toward the normal values.
HISTOLOGICAL RESULTS

I- Results by light microscope:

Control group

Pancreatic sections of control group by H&E showed normal histological feature of pancreas; the exocrine part formed of closely arranged acini with apical acidophilia and basal basophilia separated by thin septae connective tissue. Islets of Langerhans, the endocrine part, appeared as pale clusters of cells present inside the exocrine part. (Figure 3A) By toluidine blue, the pancreatic sections revealed abundant zymogen granules (ZG) (dark blue dots) in the apical part of the cells lining pancreatic acini. (Figure 3B).

Cd treated group:

H&E stained pancreatic sections of this group revealed variable degenerative changes of pancreatic acini with loss of normal lobular architecture; distorted pancreatic acini, degenerated and widely separated cells in the islets of Langerhans. Dilated blood vessels and perivascular cellular aggregations were also seen. The septae were widened. (Figure 4A, B)

Toluidine blue-stained sections showed few deeply stained apical zymogen granules in some acini, whereas other acini showed absence of these granules due to marked depletion of secretory activity of pancreatic acinar cells. (Figure 4C).

Table (1): Serum insulin (mU/L) level in different rat groups.

<table>
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<tr>
<th>Serum insulin (mU/L)</th>
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<tr>
<td>Control group</td>
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<tr>
<td>Cd treated group</td>
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<td>Cd treated group with Nigella sativa</td>
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<td>ANOVA</td>
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<td>P value</td>
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<tr>
<td>Data was presented as mean ±S.D.</td>
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<td>*Significant ≤ 0.05</td>
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<tr>
<th>Serum insulin (mU/L)</th>
<th>Control group</th>
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<th>Cd treated group with Nigella sativa</th>
<th>ANOVA</th>
<th>P value</th>
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<tr>
<td>57.3±6.01</td>
<td></td>
<td>40.1±4.96</td>
<td>55.1±6.47</td>
<td>12.85</td>
<td>0.011*</td>
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H&E stained pancreatic section of the control group showing lobules of closely packed pancreatic acini (A). The lobules are seen separated by thin incomplete connective tissue septa (S). Zymogen granules: (ZG).

Fig. 3B: A photomicrograph of toluidine blue stained pancreatic section of the control group showing lobules of closely packed pancreatic acini (A). The lobules are seen separated by thin incomplete connective tissue septa (S). Zymogen granules: (ZG).

Cadmium treated group:

Toluidine blue-stained sections showed few deeply stained apical zymogen granules in some acini, whereas other acini showed absence of these granules due to marked depletion of secretory activity of pancreatic acinar cells. (Figure 4C).
**Cadmium with Nigella sativa group**

Light microscope examination of sections of pancreas revealed some improvement of the histological picture of pancreatic acini. The pancreatic lobular architecture appeared nearly normal. However, some cells of the acini and islets of Langerhans showed mild vacuolation. (Figure 5 A) Numerous zymogen granules had been shown in the apex of many pancreatic acinar cells. However, in stained sections with toluidine blue some acinar cells revealed absence of secretory granules. (Figure 5 B).

**II-A- Electron microscopic results of the exocrine part of pancreas:**

**Control group**

Electron microscopic examination of ultrathin sections of the control group, the exocrine part of pancreas, revealed pancreatic acinar cells with basal rounded vesicular nucleus and prominent nucleoli. The acinar cells showed rough endoplasmic reticulum cisternae packed in the cytoplasm with mitochondria in-between. Electron dense zymogen granules were apically located. (Figure 6).
The pancreatic acini showed mild improvement as evidenced by appearance of normal pancreatic acinar cells. The acinar cells nuclei looked vesicular and rounded; the cytoplasm showed regularly arranged rER cisternae and variable-sized secretory zymogen granules. However, some acinar cells appeared with dilated rER and few secretory zymogen granules (Figure 8).

Cadmium treated group:

Electron microscopic examination of ultrathin sections of that group showed pancreatic acini degenerated with absent nucleus and zymogen granules. Other acinar cells showed dilated rough endoplasmic reticulum cisternae, few zymogen granules and dilated congested blood vessels (Figure 7).

Cadmium with Nigella sativa group

The pancreatic acini showed mild improvement as evidenced by appearance of normal pancreatic acinar cells. The acinar cells nuclei looked vesicular and rounded; the cytoplasm showed regularly arranged rER cisternae and variable-sized secretory zymogen granules. However, some acinar cells appeared with dilated rER and few secretory zymogen granules (Figure 8).

II-B- Electron microscopic results of the endocrine part of pancreas:

Control group

The β cells represent the predominant proportion of the pancreatic islets of Langerhans of the control rats which are present in the center of the cluster. Many insulin secretory granules with electron-dense core surrounded by an electron lucent halo are present in the cytoplasm of β-cells, the nucleus is rounded euchromatic with its nucleolus and the rough endoplasmic reticulum with its regular arranged cisternae was also shown (Figure 9 A).

Cadmium treated group:

Pancreatic specimens obtained from cadmium treated group revealed evident decrease of the characteristic insulin secretory granules and empty vesicles scattered in between congested blood capillaries. These empty vesicles are due to excessive loss of their granules in rarified cytoplasm leaving electron lucent areas. Degranulation and dilatation of rER and irregular shaped dark electron dense nucleus with dilated nuclear envelope were also remarked (Figure 9B).
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Cadmium with Nigella sativa group

B cells of Cadmium with Nigella sativa group looked nearly like control group showing many insulin secretory granules and also some empty vesicles were seen. The nucleus of beta cells is identical to control group with evident nucleolus and intact nuclear membrane. Mitochondria and rER appeared normally (Figure 9 C).

DISCUSSION

The chronic effects of irritants, in many tissues and organs are detected in a fast way by histology[13]. Cadmium has been known to increase free radical production, deteriorating the oxidation of DNA lipids and proteins and inducing a variety of pathological processes in humans and many animals[14, 15]. After acute exposure to Cd, the chief target organ is the liver. Nearly half of Cd absorbed systemically is rapidly concentrated in the liver, leading to reduction of amount of Cd delivered to other organs such as the pancreas, kidneys and testes, which are more sensitive to its toxic actions[16].

The current study evaluated the effect of NS intake in the amelioration of serum insulin level and histological changes induced by CdCl₂ on the pancreatic acinar cells and β-cells of albino rats. The hormonal assay showed a significant decrease in insulin level in rats after CdCl₂ oral administration for 3 consecutive months. This result is in consistent with the study[17] which found that exposure to Cd decreased significantly the insulin fasting serum level in mice.

It was stated[18] that Cd exposure is thought to trigger pancreatic damage resulting in a decrease in insulin production. This is based on the research[19] which states that Cd can damage β Langerhans cells in the pancreas. The subsequent decrease in insulin causes the membrane permeability of glucose to be disrupted so that glucose cannot enter the cell.
Other researchers\cite{20,21} clarified that β Langerhans cells, responsible of insulin biosynthesis/secretion in mammals, are very sensitive to ROS attack than other cell types so they are more susceptible to apoptosis. Also, they mentioned that mitochondria of β-cells can produce excessive levels of ROS with failure of the ROS defense system, resulting in oxidative stress damage of B-cells.

In this study, oral administration of CdCl$_2$ for three months induces degenerative changes of pancreatic acini with loss of normal lobular architecture, distorted pancreatic acini, degenerated and widely separated cells in the islets of Langerhans, dilated blood vessels and abundant perivascular cellular aggregates in pancreatic tissues stained with H&E. These lesions agreed with\cite{22} who found congested vessels and interstitial infiltration of mononuclear cells, intra and inter lobular duct hyperplasia and mild loss of endocrine part of the pancreas in cadmium group of rats.

The present study showed variation of zymogen granules after oral intake of CdCl$_2$ in toluidine blue stained pancreatic sections. Other investigators\cite{23} recorded the decrease number of the granular content of some acini associated with large vacuoles while other acini retained their content.

They reported that one of the major effects in pancreatitis is the unlimited release of enzymes into the interstitial space which leads to autodigestive fat necrosis by the action of lipase. Moreover, vascular damage and widening of the interstitial tissue were also frequent observations. This vascular damage will produce free radicals which activate zymogen protease in acinar cells resulting in pancreatitis.\cite{24,25}

The present study showed ultrastructural lesions of acinar cells in specimens of rat fed on CdCl$_2$, such as dilatation of RER, swollen, and damaged mitochondria as well as empty vesicles. The same findings were previously described by other authors\cite{26,27,28,29} as acinar cell necrosis. The beginning of pancreatitis and destruction of acinar cell is through activation of lysosomes and decomposition of cellular organelles in autophagosomes. This led to formation of large secretory vacuoles containing lysosomal and digestive enzymes.

Also in this study, there were a difference between the cadmium treated group and the control group regarding the nucleolus it was absent in many cells of the cadmium treated group and it was evident and even double in the protected group. Some investigators\cite{30} mentioned that nucleolus is a very dynamic structure able to rapidly change its architecture in response to various stimuli.

Cadmium could be concentrated in the pancreas and cause the change of the zinc levels. Then it resulted in the change of the expression of gene and protein and influences the functions of both endocrine and exocrine parts of the pancreas\cite{31}. Cadmium enhances the production of free radicals in pancreas\cite{32}. Pancreatic β-cells, which function in insulin biosynthesis and secretion in mammals, are at greater risk of apoptosis due to ROS attack than other cell types. The mitochondria of β-cells can generate excessive levels of ROS and with a failure of the ROS defense system, this will result in the relatively high vulnerability of β-cells to oxidative stress damage.\cite{33}

In the study\cite{34} on Cd-treated rats, degranulation, mild degeneration and apoptosis were observed in approximately 30% of β-cells of the islets of Langerhans on the 15$^{th}$ day of pregnancy. On the 20$^{th}$ day of pregnancy, the number of degenerated, necrotized, and degranulated β-cells increased. Also, another study stated\cite{35} that in Cd treated rats with 0.49mg Cd/kg/d for 30 days, B-cells of pancreatic islets had severe loss of granulation, necrosis and degeneration. Oxidative stress is an essential factor in causing insulin resistance and β-cells dysfunction through their capacity to activate stress-sensitive signaling pathways.\cite{36} Pancreatic islets cells manifest weak antioxidative enzymes so the pancreas may be more vulnerable to oxidative stress than other tissues and organs.\cite{37}

The current study showed that administration of Nigella Sativa with CdCl$_2$ exerts an important protective effect as it corrected the oxidative stresses. This finding agreed with the findings of the study\cite{38,39} which found that NS expressed a protective effect against toxic influence of cadmium on all the examined parameters in rat blood. NS oil had the option to safeguard cells against oxidative stress-induced cell injury. There are three elective clarifications for the defensive impact of NS oil against oxidative stress. They may act as direct antioxidants, block reactive oxygen species generation by inhibiting a step in the programmed cell death pathway (apoptosis), or directly cause a low level of ROS production that quickly initiates a reactive oxygen species defense system before the glutamate-induced cell death program is finished. The latter is a type of preconditioning that could be brought about by the exposure of cells to reactive oxygen species lowering material.\cite{40}

The preventive effects of NS might be the result of suppression of fat peroxidation in Cd-treated rats by its antioxidant nature. But they recommended more researches to know the exact mechanism of this modulatory effect.\cite{41}

**CONCLUSION**

In conclusion, this study shows that cadmium is a potent exocrine and endocrine disruptor which can cause damage of pancreas architecture and NS could have a protecting effect against these alterations.
RECOMMENDATION:

Cadmium could affect other cells in the islet of pancreas like alpha and delta cells. More investigations are needed to evaluate the histological and hormonal changes due to cadmium toxicity.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES


الملخص العربي

التأثير البيوكيميائي والهستولوجي لكادميوم كلوريد على بنكرياس الجرذان البيضاء البالغة وتأثير الحماية الممكنة لبذور حبة البركة

المقدمة: تقيم الدراسة الحالية الدور الوقائي لحبة البركة ضد التغيرات البيوكيميائية والنسيجية التي يسببها كادميوم في بنكرياس الجرذان.

المواد والطرق: تم تقسيم ثمانية عشر جرذًا بالغًا من جرذان ويستار البيضاء بالتساوي إلى ثلاث مجموعات.

المجموعة الضابطة، المجموعة المعالجة بالكادميوم والتي تلقته 5 مجم من كادميوم كلوريد كجم من وزن الجسم / يوم مذابة في الماء عن طريق الفم لمدة ثلاثة أشهر. وتأتي مجموعة الكادميوم مع حبة البركة مزيجًا من 1 مجم / كجم / يوم من المعلق الماني من مسحوق بذور حبة البركة و 5 مجم من الكادميوم كلوريد كجم من وزن الجسم / يوم مذاب في الماء لمدة ثلاثة أشهر متتالية. في نهاية التجربة، تم إجراء الفحص الهرموني للأنسولين. تم تحضير أنسجة البنكرياس لكل من الهيماتوكسيلين والأيوزين والتولويدين الأزرق. كما أجريت دراسة مجهرية إلكترونية لنسج البنكرياس.

النتائج: أظهرت الدراسة الحالية أن تناول كادميوم كلوريد تسبب في انخفاض معنوي في مستوى الأنسولين في الدم. تم تأكيد النتائج البيوكيميائية من خلال التحقيقات النسيجية حيث تم الكشف عن التغيرات التنكسية لأسيني البنكرياس مع فقدان المصلحة الفصية، والخلايا المتدهورة، والزوجين القمي في جزر إنجرانز H&E، والأوعية الدموية المحتقة والتسلل الخلوي حول الأوعية الدموية في أقسام البنكرياس المطليتة بالتيولودين المصبوغة باللون الأزرق القليل من حبيبات الزيموجين القمي المطلقة بعمق في بعض أسيني، بينما أظهرت أسيني أخرى عدم وجود هذه الحبيبات.

أظهر تناول حبة البركة مع كادميوم كلوريد تحسنًا ملحوظًا في مستوى الأنسولين في الدم المقاس والصور النسيجية.

الخلاصة: تسببت كادميوم كلوريد في التغيرات البيوكيميائية والنسيجية في الأجزاء الخارجية والعدل الصماء من البنكرياس في الجرذان كما يمكن أن تلعب حبة البركة دورًا وقائيًا ضد هذه التغيرات.

الكلمات المفتاحية: كادميوم كلوريد، الإجهاد التأكسدي والسامة، البنكرياس، التغيرات النسيجية، حبة البركة.