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Histomorphometric and stereological study of testicular tissue in diabetic rats following lead acetate and cinnamon treatment

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Abstract Uncontrollable acute and chronic diabetes endangers people's quality of life in both developed and underdeveloped countries. Therefore, it appears crucial to investigate diabetes and its management strategies. Following the growth of environmental contaminants such as lead, we are seeing a rise in physical illnesses. Lead is absorbed and carried into the circulatory system, where it binds to the hemoglobin of red blood cells and accessing the tissues. The utilization of herbal plants therapy is growing nowadays. Cinnamon has a significant influence in neutralizing free radicals due to its numerous antioxidant capabilities, consequently the main objective of the above study was to evaluate the antioxidant effects of cinnamon against the oxidative stress of lead acetate on alloxan-induced diabetic rats. 24 male Wistar-albino rats were selected and slaughtered after the treatment period in accordance with animal welfare guidelines. Following sample preparation, the prepared slides were inspected and the data were analyzed using SPSS software. Histomorphometrical evaluations indicated an increase in the number and area of seminiferous tubules in the diabetic and lead acetate-treated diabetic group following cinnamon administration. Furthermore, surface evaluating examinations indicated that cinnamon treatment increased the area of the seminiferous tubules while decreasing the area of the interstitial parenchyma in the diabetic group and the lead acetate-treated diabetic group.

Introduction

The insufficient production of insulin by pancreatic beta cells, commonly referred to as diabetes, leads to various health complications, including nephropathy, neuropathy, retinopathy, and gastrointestinal disorders [15, 19]. Unregulated acute and chronic high blood sugar levels not only affect the well-being of individuals but also lead to substantial health concerns and economic burdens [16, 18, 23].

According to studies, 95% of the lead that enters the body accumulates in the bone and goes through milk, sweat, and placental secretions [4, 9]. Lead-containing substances disturb biological processes by creating reactive oxygen radicals (ROS), which reduces the tissue antioxidant capacity called oxidative stress [4, 9]. Besides various modern treatments, conventional agents and herbal therapy are considered alternatives for management of diabetes.

Cinnamon, scientifically known as *Cinnamomum zeylanicum*, is one of the ancient herbs in traditional medicine [2]. Because of its high concentration of volatile fatty acids such as Cinnamaldehyde, Eugenol, and a semi-insulin-like substance known as Saphrol, cinnamon lowers triglycerides, total cholesterol, and low-

density lipoprotein [12]. Due to its numerous naturally occurring antioxidants, cinnamon has also a great free radical scavenging activity [2, 11, 18].

The aim of the present study is was to investigate the effect of cinnamon on lead acetate oxidative stress in alloxan-induced diabetic rats.

Materials and Methods

Animals

A total of 24 male Wistar albino rats weighing an average of 200 ± 20 g were purchased and kept under 12:12-h light-dark cycle, with free access to food and water and a standard temperature of 22±2°C. All laboratory technical procedures were performed according to the ethical guidelines and animal welfare standards.

Experimental design

The animals were randomly divided into four groups (each group contain six rats), as follows:

Group 1: The control group, which was fed a standard chow pellet with no treatment. Group 2: The diabetic group, which was received a single dose of alloxan (220 mg/kg, i.p.). Group 3: The diabetic group, which was received alloxan (220 mg/kg, i.p.) and lead acetate (200 ppm, orally). Group 4: The diabetic group, which was received alloxan (220 mg/kg, i.p.), lead acetate (200 ppm, orally), and cinnamon (70 mg/kg, orally). Finally, animals were euthanized with chloroform, and cardiac blood samples were taken.

Histopathological assay

After euthanasia, the right testes of the mice were removed and kept for 48 hours in 10% neutral buffered formalin 10%. A gradation of ethanol was used to dehydrate the samples. They were then embedded in paraffin after they were cleared in xylene. The samples were then cut in 0.5 µm thickness. After hematoxylin and eosin (H&E) staining, the slides were studied by an optical microscope.

Histomorphometric and Stereological analysis

The tissue slides were inspected histologically and stereoscopically using a light microscope. The quantity and diameter of seminiferous tubules in histological investigations, as well as the area of interstitial parenchyma and seminiferous tubules in stereological studies, were assessed using a micrometer.

Statistical analysis

All data analysis were presented as mean ± standard deviation and P-value < 0.05 was considered statistically significant. The statistical study was performed through one-way analysis of variance (ANOVA) and Tukey's HSD post hoc analysis was used with SPSS software (version 16).

Results

Histomorphometric evaluation

The results of Histomorphometric investigations showed a significant decrease in the number of spermatogenic tubules in diabetic rats and diabetic rats treated with lead acetate (group No. 3) as compared to the control rats. There was no significant difference between the number of spermatogenic tubules in diabetic rats treated with both lead acetate and cinnamon (group 4) when compared to the control group. The number of spermatogenic tubules in diabetic rats treated with both lead acetate and cinnamon (group 4) was higher than in diabetic animals treated with lead acetate (group 3), which indicates the improvement in spermatogenic tubules quantity after cinnamon treatment. The maximum and the minimum number of the seminiferous tubule were observed in the control group and lead acetate-treated diabetes group, respectively (Figure 1).

Histopathological evaluation showed that diabetic rats (group 2) and diabetic rats treated with lead acetate (group 3) had lower seminiferous tubule diameter (µm) than the control group. Furthermore, the diabetic group treated with lead acetate and cinnamon (group 4) showed an increase in seminiferous tubule diameter compared to the diabetic rats (group 2) and diabetic rats treated with lead acetate (group 3). These results demonstrated an improvement in seminiferous tubule diameter after treating with cinnamon. The maximum and the minimum seminiferous tubule widths were observed in the control group and lead acetate-treated diabetes group (group 3), respectively (Figure 2).

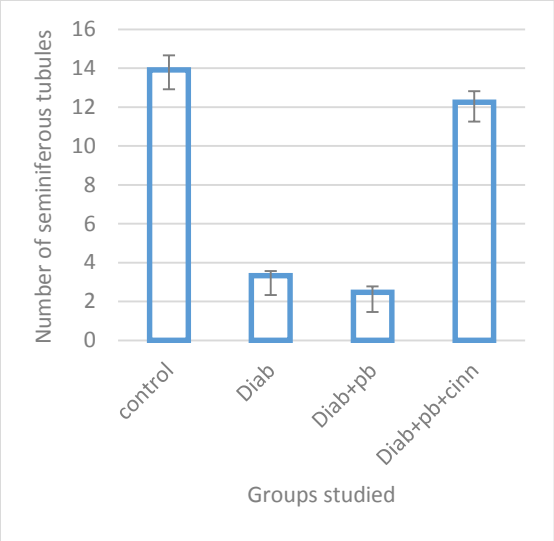

Fig 1: Diagram of the number of seminiferous tubules in the studied groups

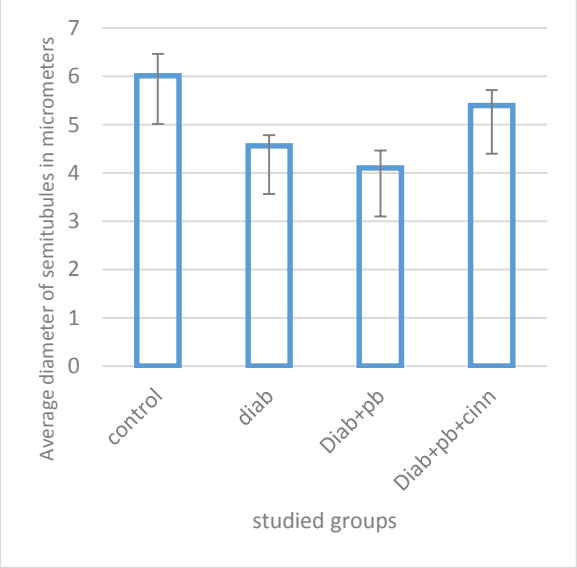

Fig 2: The average diameter of spermatogenic tubules in the studied groups

Stereological evaluation

Stereological investigations indicated a decrease in the area of spermatogenic tubules in diabetic rats treated with lead acetate, compared to the control group. Diabetic rats (group 2) and diabetic rats treated with lead acetate (group 3) had significantly smaller spermatogenic tubule area compared to those treated with cinnamon (group 4). The maximum and the minimum areas of the spermatogenic tubules were observed in the control and the diabetic rats treated with lead acetate (group 3), respectively (Figure 3).

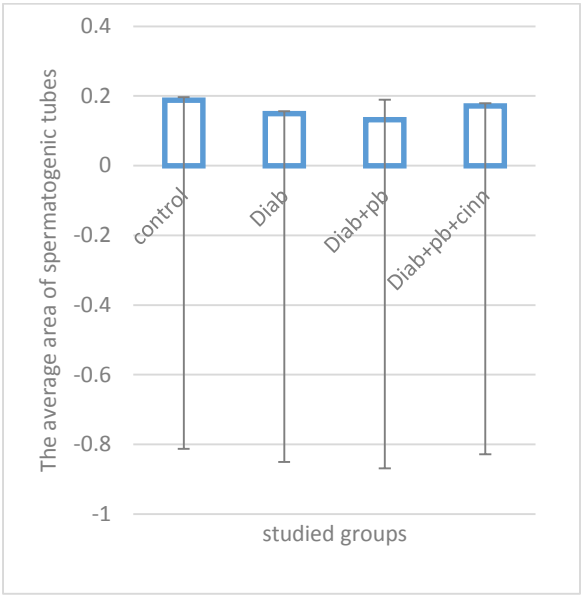

Fig 3. Graph based on stereological investigations of the average area of spermatogenic tubules

Surface examinations and stereological studies demonstrated a significant increase in the interstitial parenchyma of testicular tissue in diabetic rats (group 2) and diabetic rats treated with lead acetate (group No. 3) compared to control groups. Furthermore, the group of diabetic rats treated with lead acetate (group 3) indicated a significant increase in the interstitial parenchyma area of testicular tissue compared to the diabetic group (group 2). Figure 4 indicated notable variations in the extent of interstitial parenchyma in testicular tissue of different groups.

Discussion

In this study, histomorphometric and stereological measurements of spermatogenic tubules in diabetic rats treated with lead acetate indicated a decrease in the number and diameter of spermatogenic tubes as well as an increase in the area of interstitial parenchyma of testicular tissue. Moreover, treatment with cinnamon could alleviate lead acetate tissue damages in alloxan-induced diabetic rats.

Surface measurement studies indicated that diabetes causes decrease in the number and diameter of spermatogenic tubules as well as thicker interstitial parenchyma in rat testicular

Table 1: compares the mean diameter and number of seminiferous tubules in the studied groups

Parameters	Control*	Diabetes	Diab+pb	Diab+Pb+Cinn
Diameter of seminiferous tubules (µm)	6.0136±0.45175	4.5615±0.21987	4.1000±0.36495	5.3968±0.31875
Number of seminiferous tubules	13.9167±0.74190	3.3333±0.24254	2.4667±0.30654	12.2500±0.57499

*Control: control group, Diabetes: group of diabetic rats, Diab+pb: group of diabetic rats treated with lead acetate, Diab+Pb+Cinn: group of diabetic rats treated with lead acetate and Cinnamon

Table 2: Comparison of the average area of seminiferous tubules and testicular parenchymal tissue by stereological method in the studied groups

parameters	Control*	Diabetes	Diab+pb	Diab+Pb+Cinn
The area of seminiferous tubules	0.1877±0.00883	0.1492±0.00732	0.1316±0.00579	0.1714±0.00814
The area of interstitial parenchyma of testis	0.7209±0.00850	0.8100±0.01874	0.8508±0.00732	0.7727±0.01943

*Control: control group, Diabetes: group of diabetic rats, Diab+pb: group of diabetic rats treated with lead acetate, Diab+Pb+Cinn: group of diabetic rats treated with lead acetate and Cinnamon

tissues. Diabetes causes tissue changes in the testicular tissue as a result of free radical production and oxidative stress, including cell death, spermatogenic tubule degeneration, and spermatogenic tubule diameter reduction [3]. Reactive oxygen species (ROS) affect cells through mechanisms such as lipid oxidation and oxidative damage to nucleic acids and proteins [5].

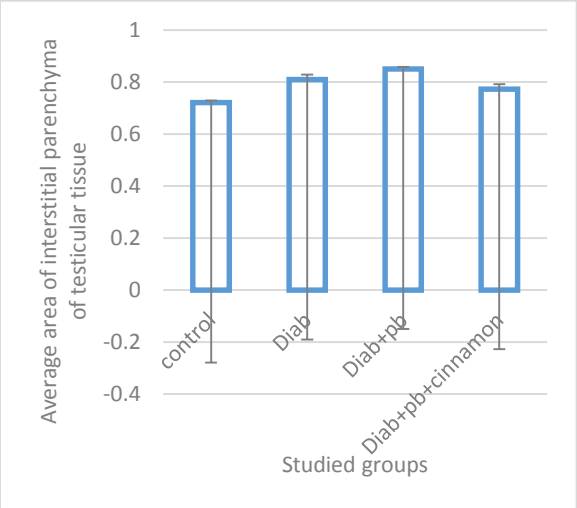

Fig 4. Graph of the data obtained from the stereological examination of the average area of interstitial parenchyma of the testicular tissue

In recent years, lead has attracted the interest of many scientists as it is now recognized as a bio-environmental pollutant. Several investigations approved the tissue degradation effects of lead and its derivative compounds in several organs of the body, including the reproductive system [1, 8]. Lead acetate destroys body tissues and promotes cell damage by inducing oxidative stress, lipid oxidation, and lowering testicular anti-oxidant capacity, including superoxide dismutase [17, 19, 22]. It is also possible that lead compounds damage the rough endoplasmic reticulum and reduce protein production and cell replication, damage to cell chromatin and the transformation of chromatin into heterochromatin. Oxidative stress, lipid peroxidation, and changes in membrane characteristics, all contribute to germ cell mortality at various stages of growth and development [1, 8].

In this study, histomorphometric and stereological measurements of diabetic rats treated with lead acetate indicated a decrease in the number and diameter of spermatogenic tubes and an increase in the area of interstitial parenchyma of testicular tissue. According to studies conducted on the effects of diabetes and lead caused by environmental pollutants in the surrounding environment on most of the body's organs, including the reproductive system, many

efforts have been made to find effective drugs to improve or reduce the complications of the disease [6, 7]. In this perspective, traditional remedies and medical herbs become more beneficial in minimizing the negative effects of chemical medications. Scientific studies have verified that cinnamon protects against the detrimental impact of oxidative agents and ROS [4, 15].

Cinnamon is full of strong antioxidants such as polyphenols and having flavonoid compounds causes the increase in glucose uptake by different cells and decreases the oxidative stress level. Oral consumption of hydroxychalcone eugenol (MHCP) leads to the normalization of glutathione peroxidase activity and an increase in reduced glutathione in cells [21]. Cinnamaldehyde, which can be found in cinnamon, has the highest anti-inflammatory properties [13].

Conclusion

The study found that diabetic rats treated with lead acetate and cinnamon had an increase in the number and diameter of spermatogenic tubules, as well as an increase in the area of the spermatogenic tubules and a decrease in the area of the interstitial parenchyma of the testicular tissue, indicating a significant improvement.

Acknowledgment

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Conflict of interest

There is no conflict of interests.

Ethical approval

All laboratory technical procedures were performed according to the ethical guidelines and animal welfare standards.

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