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Effectiveness Of Taraxacum Officinale In Rat Tissue Damage Caused By Doxorubicin

Doxorubicin'in Neden Olduğu Sıçan Doku Hasarında Taraxacum Officinale'nin Etkinliği

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Öz

Amaç: Bu çalışmada doksorubisinin neden olduğu pankreas hasarında taraxacum officinale'nin etkisini araştırmayı amaçladık.

Yöntemler: Toplam 40 adet Wistar albino rattan oluşan 4 grup oluşturuldu: Grup 1'e (kontrol grubu) hiçbir şey verilmedi. Grup 2'ye (taraxacum officinale grubu) 10 gün süreyle 100 mg/kg Taraxacum officinale verildi. Grup 3'e (doksorubisin grubu) tek doz 40 mg/kg doksorubisin verildi. Grup 4'e (doksorubisin +taraxacum officinale grubu) tek doz 40 mg/kg doksorubisin +100 mg/kg taraxacum officinale 10 gün süreyle uygulandı. Kan malondialdehit (MDA) seviyeleri ve katalaz (CAT) ve süperoksit dismutaz (SOD) aktiviteleri ölçüldü. Histopatolojik değerlendirme hematoksilin eozin boyası yardımıyla PAX 2 ve PAX 8 ifadeleri ölçülerek yapıldı.

Bulgular: Grup 4'te SOD ve CAT enzim aktiviteleri grup 3'e göre anlamlı olarak yüksekti ($p<0.05$). MDA düzeyleri grup 4'te grup 3'e göre anlamlı olarak düşüktü ($p<0,05$). Grup 3'teki doku hasarı grup 4'e göre anlamlı olarak yüksekti ($p<0.05$).

Sonuç: Taraxacum officinale, doksorubisin kaynaklı pankreas hasarını tersine çevirmede etkili görünmektedir. Bununla birlikte, büyük randomize çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Doksorubisin, taraxacum officinale, rat, pankreas

Abstract

Aim: In this study, we aimed to investigate the effect of taraxacum officinale in pancreatic damage caused by doxorubicin.

Methods: 4 groups were formed with a total of 40 Wistar albino rats: In group 1 (control group), nothing was given. In group 2 (taraxacum officinale group), 100 mg / kg Taraxacum officinale was given for 10 days. In group 3 (doxorubicin group), single dose 40 mg / kg doxorubicin was given. In group 4 (doxorubicin +taraxacum officinale group), single dose 40 mg / kg doxorubicin +100 mg/kg taraxacum officinale for 10 days were administered. Blood malondialdehyde (MDA) levels and activities of catalase (CAT) and superoxide dismutase (SOD) were measured. Histopathological evaluation was performed with the help of hematoxylin eosin stain and by measuring the expressions of PAX 2 and PAX 8.

Results: SOD and CAT enzyme activities in group 4 were significantly higher than group 3 ($p<0.05$). MDA levels were significantly lower in group 4 than group 3 ($p<0.05$). Tissue damage in group 3 was significantly higher than group 4 ($p<0.05$).

Conclusion: Taraxacum officinale appears to be effective in reversing doxorubicin-induced pancreatic injury. However, large randomized trials are required.

Keywords: Doxorubicin, taraxacum officinale, rat, pancreas

Introduction

Doxorubicin is an anthracycline derivative antineoplastic drug that has been used in cancer treatment for many years. It is known that doxorubicin has negative effects on many tissues and organs, especially the heart and liver [1]. Doxorubicin can cause congestive heart failure, which has a mortality of up to 50% [2]. It affects normal cells as well as cancer cells, therefore, potentially many tissues and organs will be more or less affected by the toxicity [3]. Aranuchalam et al reported that doxorubicin had a toxic effect on the pancreas. They demonstrated that doxorubicin inhibited blood glucose and lipid clearance and this led to lipotoxicity, glucotoxicity, and insulin resistance in rodents [4]. It has also been shown that doxorubicin disrupts the functions of adipocytes [5]. Although the mechanism of action of doxorubicin's toxicity on the pancreas is not fully known, it has been reported that it disrupts the glucose balance in the beta cells of the pancreas. It predisposes to diabetes by inhibiting insulin secretion in pancreatic beta cells in rats [6]. Doxorubicin could deteriorate the function of cytochrome P450 system [7]. Doxorubicin causes DNA damage and apoptosis by inhibiting topoisomerase activity [8]. However, the role of all these damages on pancreatic tissue toxicity are still controversial.

Taraxacum officinale (TO), also known as Dandelion, is a herbal agent belonging to the Asteraceae family. Sun et al reported that TO stimulates the immune system [9]. Previous studies have been shown that TO have antioxidative, anti-inflammatory, and neuro-protective properties [10, 11]. Therefore, we thought that TO might be effective in preventing doxorubicin-induced pancreatic injury. Moreover, to our current knowledge, there are no studies showing the effect of TO in preventing doxorubicin-induced pancreatic injury.

Materials and Methods

The study was planned and carried out in Kırşehir Ahi Evran University Faculty of Medicine, Department of Histology-Embryology. Ethical approval of the study was received from Erciyes University Animal Experiments Local Ethics Committee. The date and number of the document of ethical approval was 07.12.2022 and 22/258, respectively. 8-10 weeks old female Wistar albino rats were used and Helsinki animal rights declaration was taken into account. Animals were treated at room temperature with 12 hours of light and 12 hours of darkness. Ad libitum method was used, with free access to water and food.

Experimental design

40 rats were allocated into 4 groups:

Group 1: Control (nothing was given), (n=10)

Group 2: Taraxacum officinale group (100 mg / kg taraxacum officinale for 10 days), (n=10)

Group 3: Doxorubicin group (40 mg / kg doxorubicin single dose), (n=10)

Group 4: Doxorubicin +taraxacum officinale group (single dose 40 mg / kg doxorubicin +100 mg/kg taraxacum officinale for 10 days), (n=10)[12, 13].

Animals anesthetized by using Ketamine hydrochloride (45 mg/kg, Ketalar®, Eczacibasi, Istanbul, Turkey) and xylazin hydrochloride (5 mg/kg, Rompun®, Bayer, Leverkusen, Germany). Malondialdehyde (MDA) levels and activities of catalase (CAT) and superoxide dismutase (SOD) were measured in the blood taken from the heart of rats. All the animals were sacrificed after pancreatic tissues were removed. Tissues were kept in paraffin block until hematoxylin and eosin dye (H&E) and immunostaining.

Histopathological evaluation

Pancreatic-tissue samples fixed in 10% formalin were embedded in paraffin, cut into 4 µm sections, placed on slides and stained with hematoxylin and eosin (H&E). Slides were examined by a blinded pathologist under a light microscope (Olympus® Inc. Tokyo, Japan). A modified semi-quantitative scoring was performed for the microscopic evaluation of the pancreatitis and four categories, Grade 0: None (0%) 1: Minimal (0-5%) 2: Mild (5-25%) 3: Moderate (25-50%) 4: Severe (more than 50%) were defined. To grade the damage to the pancreas, edema, acinic cell degeneration, acinar necrosis, hemorrhage, intrapancreatic and perivascular inflammation, inflammation in the peripancreatic fat tissue were included as the parameters of the scoring system [14].

PAX2 and PAX8 expressions in the islets of Langerhans were investigated immunohistochemically. PAX2 and PAX8 expression levels were graded using the 0-3+ range. (0: no staining, 1: nuclear staining in less than 10% of Langerhans cells, 2: nuclear staining of 10-30% of Langerhans cells, 3: nuclear staining of more than 30% of Langerhans cells).

Biochemical analyses

The centrifuged blood was stored at -80 °C. The levels of MDA was measured by using the MDA kit (Cat. No: E0156Ra, Bioassay Technology Laboratory). The absorbance at 450 nm was evaluated using the ELISA method. The activities of SOD and CAT were assessed by using SOD kit (Cat. No: EIASODC, Thermofisher Scientific) and CAT kit (Cat. No: ab83464, Abcam).

Statistical analysis

Statistical Package for the Social Sciences (22.00 SPSS Inc., Chicago, IL) was used for statistical analyses. One-way ANOVA test and Post hoc Tukey HSD multiple comparison test were used for levels of MDA and NO. Tissue damage scores were compared by Kruskal Wallis test. Evaluation of caspases was determined by Fisher's Exact Test as p value. p value < 0.05 was accepted as statistically significant.

Results

The MDA levels were higher in doxorubicin group than doxorubicin +taraxacum officinale group, activities of SOD and CAT were lower in doxorubicin group than doxorubicin +taraxacum officinale group, and these differences were found to be statistically significant ($p < 0.05$) **(Table 1).**

Table 1. Distribution of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) in experimental groups.

Groups (n = 10)	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)
Control	3.10 ± 0.21	73.41± 14.62	88 ± 15.63
Taraxacum officinale (100 mg/kg)	4.91 ± 0.33	52± 9.55	60.82± 11.34
Doxorubicin (40 mg/kg)	13.27 ± 1.96*	28 ± 4.52*	30.25 ± 6.15*
Doxorubicin+Taraxacum officinale(100 mg/kg+40mg/kg)	7.46± 1.19*	39.18± 6.77*	50.94± 10.48*

MDA means malondialdehyde, SOD means superoxide dismutase, CAT means catalase

Data are presented as mean ± SD.

* Significant difference ($p < 0.05$) between groups 2 and 3.

The histopathologic damage in pancreatic tissue was significantly higher in doxorubicin group than doxorubicin +taraxacum officinale group, too ($p < 0.05$) **(Table 2).**

Table 2. Distribution of histopathologic findings.

Groups (n = 10)	Edema	Acinic cell degeneration	Acinar necrosis	Hemorrhage	Intrapancreatic and perivascular inflammation	Fat tissue inflammation	PAX2	PAX8
Control	2	0	0	0	0	0	0	3
Taraxacum officinale (100 mg/kg)	2	0	0	1	1	1	0	3
Doxorubicin (40 mg/kg)	2	2	1*	2	1	2	0	1*
Doxorubicin+Taraxacum officinale(100 mg/kg+40mg/kg)	2	1*	0*	2	1	2	0	2*

- Significant difference ($p < 0.05$) between groups 2 and 3.

A modified semi-quantitative scoring was performed for the microscopic evaluation of the pancreatitis and four categories, Grade 0: None (0%) 1: Minimal (0-5%) 2: Mild (5-25%) 3: Moderate (25-50%) 4: Severe (more than 50%) were defined.

In the control and taraxacum officinale groups, histological structure of pancreatic acini and the morphologic appearance of the parenchymal tissues were similar and normal **(Figure 1A and 1B).** There was necrosis in pancreatic tissue and degenerative changes were seen in zymogen granules in doxorubicin group **(Figure 1C).** The parameters demonstrating the damage such as edema, acinic cell degeneration, acinar necrosis, hemorrhage, intrapancreatic and perivascular inflammation, inflammation in the peripancreatic fat tissue were prominent in doxorubicin group than other groups. Although the injury was lesser, there was mild focal reactive changes and single cell necrosis in acinic cells in the doxorubicin +taraxacum officinale group **(Figure 1D).**

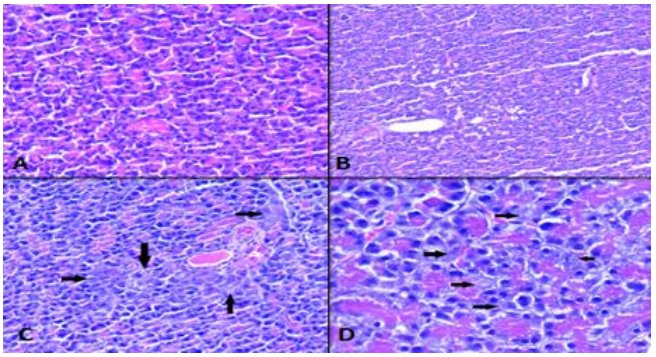

Figure 1. A) Histological structure of pancreatic acini in pancreatic tissue of rat from control group (H&E, x200). **B)** Vacuolar appearance in acinic cells in a focal area in the pancreatic tissue of a rat from the Taraxacum officinale group, no obvious degenerative findings (H&E, x200).**C)** Acinic cells in the pancreatic tissue of the Doxorubicin applied rat go to necrosis in a wide area, besides, loss and degenerative changes in zymogen granules are seen in most cells (black arrows) (H&E, x200). **D)** Single cell necrosis and mild focal reactive changes (black arrows) in acinic cells at high magnification in pancreatic tissue of a rat from Doxorubicin+Taraxacum officinale group (H&E, x400)

No staining was observed in any group of Langerhans cells with PAX2. PAX8 showed similar expression characteristics in most islets of langerhans in control and taraxacum officinale group **(Figure 2A and 2B).** In doxorubicin group, there were staining losses with PAX8 **(Figure 2C).** In the doxorubicin +taraxacum officinale group, a staining similar to the control group was detected **(Figure 2D).**

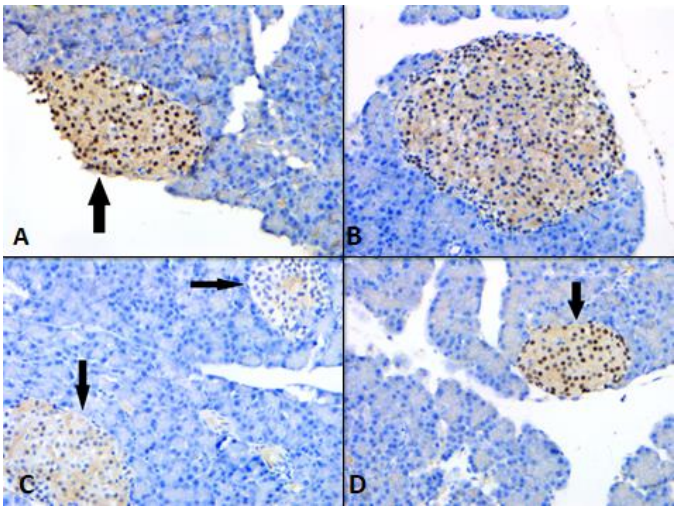

Figure 2. A) Near diffuse strong nuclear PAX8 immunoreactivity in rat langerhans cells from the control group (black arrow) (x200). **B)** Diffuse and strong staining with PAX8 in langerhans cells in the pancreatic tissue of the rat from the Taraxacum officinale group (x200). **C)** Significant loss of PAX8 immunoreactivity in Langerhans cells in group of Doxorubicin (black arrows) (x200). **D)** PAX8 expression (black arrow) in the Doxorubicin+Taraxacum officinale group, similar to the control group (x200)

Discussion

Doxorubicin inhibits topoisomerase 2, disrupts cross-linking and causes DNA damage. For this reason, Doxorubicin, which is used in cancer treatment, adversely affects normal cells as well as cancerous cells [15]. In the past, many studies have been conducted to show the toxic effects of doxorubicin, especially on the liver and heart [16, 17]. In these papers,the underlying possible mechanisms of the toxicity were reported as increased free radicals and and lipid peroxidation. The first study investigating the effect of doxorubicin on pancreatic Langerhans cells was done by Deleers et al. In that study, it was shown that Doxorubicin inhibits insulin release [6]. In this study, we investigated the effect of TO on doxorubicin induced pancreas toxicity. According to our findings, doxorubicin led to an increase in MDA levels and a decrease at SOD and CAT activities. Besides, tissue damage was more prominent in doxorubicin group. Moreover, addition of TO reversed doxorubicin-induced biochemical and histopathological damage. This was the first study indicating the effect of TO to reverse the harmful effect of doxorubicin on pancreatic tissue.

TO is a natural herbal compound and has been utilized to treat many illnesses such as gout, diabetes mellitus, diarrhea and liver disease [18]. The phenolic component in its content is responsible from the clinical efficacy. Antioxidant, antiinflammatory, and antibacterial properties of the substance might provide the protective effect [19]. Thus, we hypothesized that this antioxidant effect could be useful to reverse the pancreas injury due to doxorubicin.

In this study, doxorubicin led to a deterioration in biochemical parameters and cause histopathological damage. Addition of TO resulted in a significant improvement in both biochemical and pathological markers. Similar results were obtained when immunostaining with PAX8 was performed. Pax 2 and Pax 8 are located on the long arm of chromosome 10. Pax 2 and Pax 8 protect the cell from cell death during cellular stress. Pax 2 and Pax 8 gene expression has been shown to increase during oxidative stress. This is protective against cell death. In this study, we showed that parenchymal destruction caused by amiodarone was reversed with astaxanthin with the help of pax 2 immunohistochemical staining.The lower expression of PAX 8, especially in doxorubicin group, made us think that doxorubicin may have caused dysfunction in the development and function of pancreatic endocrine cells. In the doxorubicin+taraxacum officinale group, the expression of PAX 8 was normal and we thought that TO reversed the negative effect of doxorubicin. However, molecular studies are needed to support this proposition.

The limitations of our study were the small number of subjects and the possibility of variation when the study was adapted to humans.

In conclusion, our results showed that TO diminishes pancreatic injury and may be convenient in the treatment and management of the oxidative stress induced by doxorubicin. However, large prospective randomized trials are necessary to evaluate the efficacy of TO on the pancreatic injury due to doxorubicin.

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