

## Efficacy of *Taraxacum officinale* in liver damage caused by doxorubicin in rats

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### Abstract

**Background/Aim:** The use of doxorubicin is limited due to its toxic effects on normal cells. A substance containing antioxidant properties, such as *taraxacum officinale*, would be useful in preventing doxorubicin toxicity. This study aimed to evaluate the effect of *taraxacum officinale* on doxorubicin-induced damage in the rat liver.

**Methods:** Forty Wistar albino rats were allocated into four groups. In group 1 (control group), no treatment was given. In group 2 (*Taraxacum officinale*, group T), 100 mg/kg *Taraxacum officinale* was administered via the gavage route for 10 days. In group 3 (doxorubicin, group D), a single intraperitoneal dose of 40 mg/kg doxorubicin was given. In group 4 (doxorubicin + *Taraxacum officinale*, group D+T), a single intraperitoneal dose of 40 mg/kg doxorubicin was administered on the eighth day, and 100 mg/kg *Taraxacum officinale* was administered for 10 days. Blood malondialdehyde (MDA) levels and the activities of catalase (CAT) and superoxide dismutase (SOD) were measured. Histopathology was assessed by examining preparations of hepatic tissue with light microscopy and immunohistochemistry.

**Results:** MDA levels were significantly higher, and the activities of SOD and CAT were lower in group D than in group D+T ( $P=0.04$ ). Tissue damage was significantly higher in group D than in group D+T ( $P=0.03$ ).

**Conclusion:** Our short-term results indicate that oxidative stress could be responsible for the damage to liver tissue due to doxorubicin, and *Taraxacum officinale* might reverse these harmful effects.

**Keywords:** doxorubicin, toxicity, *taraxacum officinale*, rat, liver

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### Ethics Committee Approval

The study was approved by the Erciyes University  
Animal Experiments Local Ethics Committee  
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### Conflict of Interest

No conflict of interest was declared by the  
authors.

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## Introduction

Doxorubicin, an anthracycline derivative antineoplastic, has toxic effects on many tissues and organs, especially the liver [1]. Although doxorubicin is widely used to treat malignancies, its usage is limited due to its harmful effects on normal cells [2]. While doxorubicin is primarily cardiotoxic, it also has severe effects on the liver, which is mainly responsible for its clearance and excretion via bile [3]. The exact mechanism of liver damage remains controversial, with apoptosis, oxidative stress, and inflammation being the most commonly suggested underlying factors responsible for the hepatotoxic effect [4]. Additionally, doxorubicin forms a complex by interacting with cell DNA [5]. Wang et al. [6] reported that 40% of patients receiving doxorubicin experienced liver toxicity. Doxorubicin increases reactive oxygen species and free radicals, which affect the cell membrane in liver tissue, causing lipid peroxidation and inflammation [7,8]. No substance is known to have been clinically proven to reverse liver damage caused by doxorubicin.

*Taraxacum officinale* (TO) is a herbal substance that belongs to the *Asteraceae* family. It has been suggested that TO has immunostimulating and cancer-protective properties [9]. TO has been used to treat liver diseases and diabetes mellitus [10]. TO exhibits antioxidant properties dependent on the phenolic compounds in its content [11]. Although TO has been widely used worldwide for years, its hepatoprotective effect is poorly understood. Moreover, to the best of our knowledge, no study demonstrates the effect of TO on liver toxicity caused by cisplatin.

## Materials and methods

Forty female Wistar albino rats weighing 150–220 g were included in the study. The animals were fed a standard regimen with free access to water and food and were kept under standard conditions at a temperature of 20–22 °C. Doxorubicin was obtained from a drugstore, and TO was purchased from a herbal medicine store. Ethical approval for the study was obtained from the Erciyes University Animal Experiments Local Ethics Committee, with the document of ethical approval dated 07.12.2022 and numbered 22/258.

The animals participating in the study were divided into four groups: Group 1 (control group) received nothing, group 2 (*Taraxacum officinale*, group T) was given 100 mg/kg TO for 10 days, group 3 (doxorubicin, group D) received a single dose of 40 mg/kg doxorubicin, and group 4 (doxorubicin+TO, group D+T) received a single intraperitoneal dose of 40 mg/kg doxorubicin and 100 mg/kg TO.

The animals were anesthetized using ketamine hydrochloride (45 mg/kg, Ketalar, Eczacıbasi, Istanbul, Turkey) and xylazine hydrochloride (5 mg/kg, Rompun, Bayer, Leverkusen, Germany). Blood samples were taken by entering the heart with a needle, and then cervical dislocation was performed for euthanasia. The liver tissues of the rats were removed.

The levels of blood MDA and activities of SOD and CAT were analyzed using a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). MDA levels were detected using the

thiobarbituric acid test [12]. SOD and CAT enzyme activities were measured based on previous studies [13,14].

### Macroscopic evaluation of liver

The liver tissue was fixed in 10% formalin, and all specimens were sliced along their long axis and kept for microscopy.

### Histopathologic evaluation of liver

The tissues were embedded in paraffin blocks and sliced to a thickness of 4 micrometers. Staining was performed using hematoxylin and eosin (H&E) dye. Immunohistochemical staining was also performed for p53 and c-kit (CD117). The degree of damage was determined based on histopathological scoring according to the highest area, using the following semi-quantitative analysis: 0: None (0%), 1: Minimal (0–10%), 2: Mild (10–30%), 3: Moderate (30–50%), 4: Severe (more than 50%). Parameters were scored accordingly, using the following parameters: hepatocyte damage (cellular changes), disorganization in the hepatic cords, inflammation, congestion, hemorrhage, fibrosis, and necrosis. CD117 expression levels were graded on a 0–3+ range: 0: no staining, 1: less than 10% membranous/cytoplasmic staining in hepatocytes, 2: 10–30% membranous/cytoplasmic staining in hepatocytes, and 3: membranous/cytoplasmic staining of more than 30% of hepatocytes. p53 expression levels were also graded on a 0–3+ range: 0: no staining, 1: nuclear staining of less than 10% of hepatocytes, 2: nuclear staining of 10–30% of hepatocytes, and 3: nuclear staining of more than 30% of hepatocytes.

### Statistical analysis

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, version 22.00) was used for statistical analyses. The levels of blood MDA and activities of SOD and CAT were analyzed using a one-way analysis of variance (ANOVA) test. Tissue damage scores were compared using the nonparametric chi-square test. A *P*-value less than 0.05 was considered statistically significant.

## Results

The biochemical parameters are presented in Table 1. The MDA levels in group D were significantly higher than those in group D+T (18.41 [2.29] vs. 12.65 [1.58]; *P*=0.04). In contrast, both SOD and CAT enzyme activities were significantly lower in group D than in group D+T (*P*=0.03).

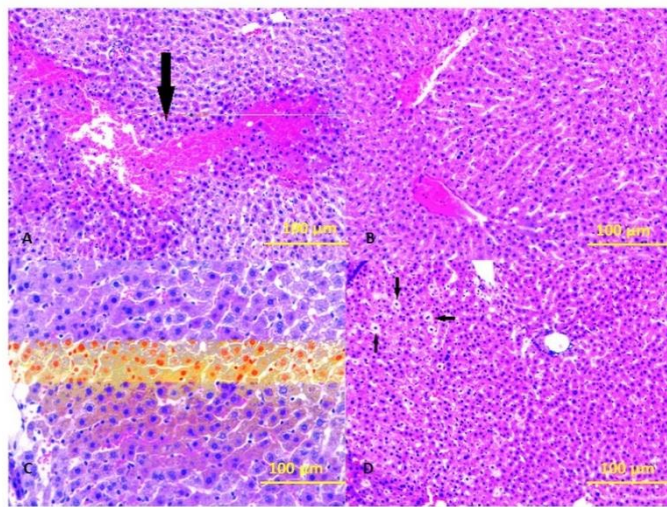
Table 1: Distribution of malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) in the experimental groups.

Groups (n=10)	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)
Control	7.22 (0.42)	64 (7.2)	78.24 (6.47)
Taraxacum officinale (100 mg/kg)	7.73 (0.44)	59 (4.56)	80.53 (8.83)
Doxorubicin (40 mg/kg)	18.41 (2.29)*	32.01 (3.5)*	41.38 (4.66)*
Doxorubicin+Taraxacum officinale (100 mg/kg+40mg/kg)	12.65 (1.58)*	47.24 (4.08)*	62.57 (6.94)*

MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase. Data are presented as mean (SD). \*Significant difference (*P*<0.05) between groups 2 and 3.

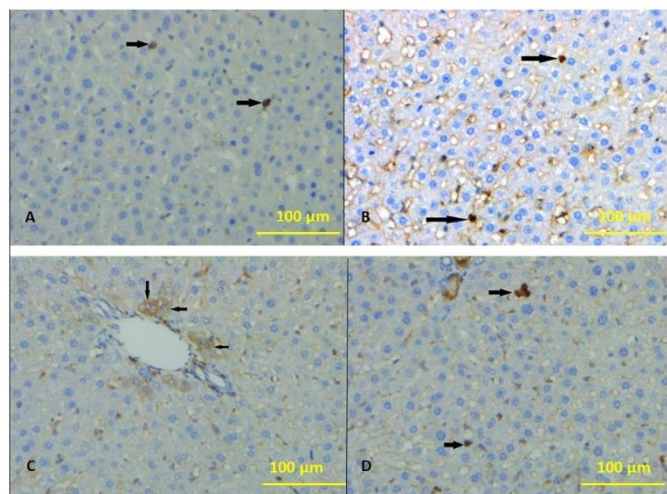
The morphology and parenchymal structures of liver tissues in the control and T groups were normal and intact, as shown in Figures 1A and 1B. In the group D, hepatocytes and parenchymal tissue exhibited signs of injury, as depicted in Figure 1C. Group D showed more severe liver damage than the other groups. Compared to group D, the group D+T showed a decrease in hepatocytes with disorganization of the hepatic cords and damage, as illustrated in Figure 1D.

Figure 1: A) Normal histological architecture in the liver of rats from the control group (H&E, x100). B) Focal mild degenerative findings in the rat liver from group T (H&E, x200). C) Hepatocytes with degenerative changes in the group D; There is granular appearance in the cytoplasm, darkening of the nucleus, binucleolization, nuclear contour irregularity, slight focal distortions of the trabeculae, as well as single-cell necrosis (arrows) (H&E, x200). D) Although cellular degenerative changes are less in hepatocytes in the group D+T than group D, vacuolar degeneration is present in some hepatocyte cytoplasm (arrows) (H&E, x100).



There was no significant difference observed in p53 immunostaining among the groups. When hepatocytes and liver parenchyma were examined, no difference was observed between the groups in terms of p53 staining. After CD117 immunostaining, histopathological damage was re-evaluated. Mild cytoplasmic immunorexpression of CD117 was observed in the group D, as shown in Figure 2.

Figure 2: A) Immunorexpression in two cells with CD117 in the liver of a rat from the control group (x200). B) Rare cell immunorexpression with CD117 in the liver of a rat from group T, similar to the control group (x200). C) Mild cytoplasmic immunorexpression by CD117 in a focal area in the liver of a group D rat (x200). D) Mild cytoplasmic immunorexpression with CD117 in relatively fewer cells in the liver of a rat from the group D+T compared to the group D (x200).



The histopathologic damage scores were significantly higher in the group D than in the group D+T, and the difference was statistically significant ( $P=0.03$ ) (Table 2).

Table 2: Distribution of histopathologic findings.

Groups (n=10)	Hepatocyte damage	Disorganization of the Hepatic Cords	Inflammation	Congestion	Hemorrhage	Necrosis	p53	CD117 Expression
Control	0	0	0	1	0	0	0	0
Taraxacum officinale (100 mg/kg)	0	0	1	1	0	0	0	0
Doxorubicin (40 mg/kg)	2*	1	1	1	1*	0	0	1
Doxorubicin+Taraxacum officinale(100 mg/kg+40mg/kg)	1*	0*	1	1	0	0	0	0*

\* Significant difference ( $P<0.05$ ) between groups D and group D+T. Histopathological scoring was done by determining the highest area. Four categories (0= None, 1=Minimal, 2=Mild, 3=Moderate, 4=Severe) were determined by making a semi-quantitative analysis, and the parameters were scored accordingly.

## Discussion

Doxorubicin is commonly used to treat various malignant diseases, including solid tumors and blood cancers. However, its effectiveness is limited due to its negative impact on both normal and cancerous cells [15]. Bilgic et al. [16] demonstrated that a single dose of doxorubicin could lead to acute liver damage. Therefore, in our study, we also administered a single dose of doxorubicin. Our results indicated that doxorubicin increased MDA levels and decreased SOD and CAT activities in the treated group. However, we hypothesized that TO could mitigate these effects by reducing MDA levels and increasing SOD and CAT activities. Our study concluded that TO could improve the biochemical and histopathological damage caused by doxorubicin. To the best of our knowledge, this study is the first to demonstrate the positive impact of TO on the liver's adverse reactions to doxorubicin.

Prasanna et al. [17] have reported that oxidative stress is the primary cause of doxorubicin-induced liver damage. As a result of doxorubicin-induced oxidative stress, electrons are lost from oxygen, leading to the production of superoxide radicals and reactive oxygen species (ROS). The elevated levels of ROS, in turn, cause an increase in lipid peroxidation, ultimately causing damage to hepatocytes and the liver [18]. Our study found that the D group had more hepatocyte damage indicators, such as inflammation and hemorrhage, than other groups. Furthermore, the D group showed more pronounced biochemical parameters indicating liver damage. However, the addition of TO was observed to reverse these adverse effects.

TO is a plant commonly found in nature, and its leaves can be eaten raw or used in tea, while its roots can be cooked and consumed. For centuries, TO has been a key component in traditional medicine for treating gout, diarrhea, and liver ailments [19]. It has been shown that TO can prevent oxidative stress in neurons, and it is believed that the protective effect is due to its phenols and hydroxycinnamic acid components [20]. Many studies have also demonstrated that TO possesses antioxidant, anti-inflammatory, antibacterial, and anticancer properties [21,22]. Therefore, we hypothesized that the antioxidant properties of TO could effectively prevent liver damage caused by doxorubicin.

In our study, we observed that TO increased the activities of SOD and CAT while reducing the levels of MDA. Histopathological analysis confirmed the protective effects of TO. The scores indicating tissue damage were significantly lower in the D+T group than in the D group. While there was no significant difference in p53 immunohistochemical staining, CD117 dye showed similar findings as H&E.



## Limitations

Our study has certain limitations, such as the small sample size and the fact that animal experiments may not perfectly replicate the effects seen in humans.

## Conclusions

In conclusion, our study found that TO can reduce hepatic injury and may be a useful treatment option for managing the oxidative stress caused by doxorubicin. We hope that our findings will inspire further research in this field. However, large prospective randomized trials are necessary to evaluate the efficacy of TO in preventing hepatic injury caused by doxorubicin.

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## References

- Nam J, Son S, Ochyl LJ, Kuai R, Schwendeman A, Moon JJ. Chemo-photothermal therapy combination elicits anti-tumor immunity against advanced metastatic cancer. *Nat Commun.* 2018;9:1074.
- Roychoudhury S, Kumar A, Bhatkar D, Sharma NK. Molecular avenues in targeted doxorubicin cancer therapy. *Future Oncol.* 2020;16:687-700.
- Johnson PJ, Dobbs N, Kalayci C, Aldous MC, Harper P, Metivier EM, et al. Clinical efficacy and toxicity of standard dose adriamycin in hyperbilirubinaemic patients with hepatocellular carcinoma: relation to liver tests and pharmacokinetic parameters. *Br J Cancer.* 1992;65:751.
- Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, et al. Doxorubicin pathways: Pharmacodynamics and adverse effects. *Pharm Genom.* 2011;21:440-6.
- Pfützer L, Moser C, Gegenfurtner F, Arner A, Foerster F, Atzberger C, et al. Targeting actin inhibits repair of doxorubicin-induced DNA damage: A novel therapeutic approach for combination therapy. *Cell Death Dis.* 2019;10:1546-9.
- Wang Y, Mei X, Yuan J, Lu W, Li B, Xu D. Taurine zinc solid dispersions attenuate doxorubicin-induced hepatotoxicity and cardiotoxicity in rats. *Toxicol Appl Pharmacol.* 2015;289:1-11.
- Barakat BM, Ahmed HI, Bahr HI, Elbahaie AM. Protective Effect of Boswellic Acids against Doxorubicin-Induced Hepatotoxicity: Impact on Nr2/HO-1 Defense Pathway. *Oxid Med Cell Longev.* 2018;8:296451.
- Nagai K, Oda A, Konishi H. Theanine prevents doxorubicin-induced acute hepatotoxicity by reducing intrinsic apoptotic response. *Food Chem Toxicol.* 2015;78:147-52.
- Sun AS, Ostadal O, Ryznar V, Dulik I, Dusek J, Vaclavik A, et al. Phase I/II study of stage III and IV non-small cell lung cancer patients taking a specific dietary supplement. *Nutr Cancer.* 1999;34:62-9.
- Schütz K, Carle R, Schieber A. Taraxacum-a review on its phytochemical and pharmacological profile. *J Ethnopharmacol.* 2006;107:313-23.
- Diaz K, Espinoza L, Madrid A, Pizarro L, Chamy R. Isolation and identification of compounds from bioactive extracts of Taraxacum officinale (Dandelion) as a potential source of antibacterial agents. *Evidence Based Complementary and Alternative Medicine* 2018;2706417:1-8.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351-8.
- Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem.* 1974;47:469-74.
- Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121-6.
- Rivankar S. An overview of doxorubicin formulations in cancer therapy. *J Cancer Res Ther.* 2014;10:853-8.
- Bilgic S, Ozgocmen M. The protective effect of misoprostol against doxorubicin induced liver injury. *Biotech Histochem.* 2019;94:583-91.
- Prasanna PL, Renu K, Gopalakrishnan AV. New molecular and biochemical insights of doxorubicin-induced hepatotoxicity. *Life Sci.* 2020;250:117599.
- Wang M, Zhang X, Xiong X, Yang Z, Li P, Wang J, et al. Bone Marrow Mesenchymal Stem Cells Reverse Liver Damage in a Carbon Tetrachloride-induced Mouse Model of Chronic Liver Injury. *In Vivo.* 2016;30:187-93.
- Ren YS, Zheng Y, Duan H, Lei L, Deng X, Liu XQ. Dandelion polyphenols protect against acetaminophen-induced hepatotoxicity in mice via activation of the Nrf-2/HO-1 pathway and inhibition of the JNK signaling pathway. *Chin J Nat Med.* 2020;18:103-13.
- Liu Q, Chen Y, Shen C, Xiao Y, Wang Y, Liu Z. Chicoric acid supplementation prevents systemic inflammation-induced memory impairment and amyloidogenesis via inhibition of NF- $\kappa$ B. *FASEB J.* 2017;31:1494-507.
- Im DY, Lee KI. Antioxidative and antibacterial activity and tyrosinase inhibitory activity of the extract and fractions from Taraxacum coreanum Nakai. *Korean J Med Crop Sci.* 2011;19:238-45.
- Park MS, So JS, Bahk GJ. Antioxidative and anticancer activities of water extracts from different parts of Taraxacum coreanum Nakai cultivated in Korea. *J Korean Soc Food Sci Nutr.* 2015;44:1234-40.