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Comparative Antioxidant Effects of Allopurinol and Febuxostat in Doxorubicin-Treated Rats

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Abstract

Background and objectives: Doxorubicin (DOX) suffers from the limitation of oxidative and nitrosative stress-derived cardiac toxicity during clinical use. Allopurinol, febuxostat, and other xanthine oxidase inhibitors are antioxidants that may mitigate oxidative injury caused by DOX. The objective of this study was to compare the effects of allopurinol and febuxostat on the biomarkers of oxidative stress and nitrosative stress in a rat model for doxorubicin-induced oxidative damage.

Methods: Forty adult rats were randomly allocated to four groups (n = 10/group): Control, doxorubicin-treated (D), doxorubicin + allopurinol-treated (A), and doxorubicin + febuxostat-treated (F). Doxorubicin was used to induce oxidative stress, and allopurinol and febuxostat were given in combination as protectors. Serum concentrations of malondialdehyde (MDA), nitric oxide (NO), 3-nitrotyrosine (3-NT), and total antioxidant status (TAS) were determined by ELISA-based assays. Histopathological examination of the aorta was also performed. Data were analyzed statistically using ANOVA with appropriate post hoc testing at $p < 0.05$ significance.

Results: Doxorubicin administration significantly increased serum MDA, TAS, NO, and 3-NT concentrations and caused marked histopathological damage in aortic tissues. The elevation of NO in the doxorubicin group may represent a compensatory response to oxidative stress aimed at maintaining vascular homeostasis, as nitric oxide normally functions as an important endogenous vasodilator and antioxidant. Both allopurinol and febuxostat significantly reduced MDA levels compared with the doxorubicin group. Allopurinol also significantly decreased NO concentrations, whereas febuxostat had no significant effect. Neither treatment significantly improved TAS or 3-NT concentrations. Histopathological examination demonstrated attenuation of doxorubicin-induced cardiovascular injury by both drugs, with febuxostat exhibiting superior preservation of aortic architecture. **Conclusion:** Both allopurinol and febuxostat exerted protective effects against doxorubicin-induced oxidative stress, primarily by reducing lipid peroxidation. Allopurinol additionally decreased nitric oxide concentrations. Despite limited differences in circulating biomarkers, febuxostat provided superior histopathological protection.

التأثيرات المضادة للأكسدة المقارنة للألوبورينول والفيبوكسوستات في الجرذان المعالجة بالدوكسوروبيسين

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الخلاصة:
الدوكوروبسين يعاني من مشكلة سمية القلب الناتجة عن الإجهاد التأكسدي والنتراتي أثناء الاستخدام السريري. الألوبورينول، فيبوكسوستات، ومثبطات أوكسيداز الزانثين الأخرى هي مضادات أكسدة قد تقلل من الضرر التأكسدي الناتج عن DOX. هدف هذه الدراسة كان مقارنة تأثير الألوبورينول وفيبوكسوستات على مؤشرات الإجهاد التأكسدي والنتراتي في نموذج جرذي لضرر التأكسد الناتج عن الدوكوروبسين. تم تقسيم أربعون فأرًا بالغًا عشوائيًا إلى أربع مجموعات (10 لكل المجموعة): المجموعة الضابطة، المجموعة المعالجة بالدوكسوروبيسين (D)، المجموعة المعالجة بالدوكسوروبيسين + الألوبيرينول (A)، والمجموعة المعالجة بالدوكسوروبيسين + الفيبكسوستات (F). تم استخدام الدوكسوروبيسين لإحداث الإجهاد التأكسدي، وتم إعطاء الألوبيرينول والفيبكسوستات معًا كمواد واقية. تم تحديد تركيزات المصل من المالونديالديهيد (MDA)، وأكسيد النيترك (NO)، وثلاثي نيتروتيروسين (NT-3)، والحالة المضادة للأكسدة الإجمالية (TAS) باستخدام اختبارات ELISA. كما تم إجراء الفحص النسيجي للأبهر. تم تحليل البيانات إحصائيًا باستخدام تحليل التباين ANOVA مع اختبار لاحق مناسب عند مستوى دلالة $p < 0.05$. أدى إعطاء دوكسوروبيسين إلى زيادة كبيرة في تركيزات MDA و TAS و NO و NT-3 في المصل وتسبب في تلف نسيجي واضح في أنسجة الشريان الأورطي. قد تمثل زيادة NO في مجموعة دوكسوروبيسين استجابة تعويضية للإجهاد التأكسدي تهدف للحفاظ على التوازن الوعائي، حيث يعمل أكسيد النيتريك عادة كمتسع وعائي داخلي مهم وكمضاد أكسدة. كل من الألوبورينول والفيبوكسوستات قللا بشكل كبير من مستويات MDA مقارنة بمجموعة دوكسوروبيسين. كما قام الألوبورينول بتقليل تركيزات NO بشكل كبير، في حين لم يكن للفيبوكسوستات تأثير ملحوظ. ولم يحسن أي من العلاجين بشكل كبير تركيزات TAS أو NT-3. أظهرت الفحوصات النسيجية تخفيف الضرر القلبي الوعائي الناتج عن دوكسوروبيسين بواسطة كلا الدوائين، مع تفوق الفيبوكسوستات في الحفاظ على بنية الشريان الأورطي .كل من الألوبورينول والفبوكسوستات أظهرا تأثيرات وقائية ضد الإجهاد التأكسدي الناتج عن الدوكسوروبيسين، بشكل رئيسي عن طريق تقليل تأكسد الدهون. كما أن الألوبورينول قلل أيضًا من تركيزات أكسيد النيتريك. على الرغم من الاختلافات المحدودة في المؤشرات البيوكيميائية الدورية، إلا أن الفبوكسوستات قدّم حماية أفضل من الناحية النسيجية.

الكلمات المفتاحية: الإجهاد التأكسدي، ألوبورينول، فيبوكسوستات، الإجهاد النيتروزياتي.

INTRODUCTION

Xanthine oxidase (XO) is a significant enzymatic source of reactive oxygen species (ROS), producing superoxide radicals and hydrogen peroxide during purine metabolism. These ROS participate in normal redox-sensitive signalling pathways; however, when generated in excess, they may contribute to oxidative stress and tissue dysfunction associated with conditions such as inflammation and ischemia–reperfusion injury[1]. Consequently, studies regarding antioxidants and cardio-protectants have paid more and more attention to XO inhibitors[2]. Allopurinol and the drug-directed febuxostat are clinically relevant Xanthine Oxidase (XO) inhibitors, which have been approved for the treatment of hyperuricemia and gout. There are significant differences in the pharmacology of these two agents. Allopurinol, as well as oxypurinol (the active metabolite of allopurinol), both possess direct free radical scavenging activity in addition to an antioxidative capacity (inhibiting XO), while febuxostat is a larger, more selective, and stronger non-purine derivative of a xanthine oxidase inhibitor (weak intrinsic antioxidant activity only)[3].

Although febuxostat is a powerful and efficient urate-lowering medication, questions have been raised regarding its cardiovascular safety. Multiple clinical trials have shown that febuxostat is associated with higher cardiovascular mortality than allopurinol[4]. The mechanisms responsible for these adverse coronary events, however, are not well understood. One potential explanation for this is that potent uric acid suppression, a principal endogenous plasma antioxidant, can limit whole-body antioxidant defence and disrupt redox homeostasis[5,6].

Doxorubicin is a potent, widely used anthracycline chemotherapeutic agent for solid and hematological malignancies. Its use in clinical practice is limited by serious toxicities, especially cardiotoxicities, which correlate greatly with oxidative and nitrosative stress. Redox cycling of doxorubicin occurs intra-mitochondrially and produces excess reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide. Simultaneously, inflammatory activation and inducible NO production promote accumulation of ONOO−, a powerful oxidant that can initiate protein nitration, leading to cell injury[7,8].

3-NT is regarded as a potential marker of nitrosative stress and nitrated proteins caused by peroxynitrite. More 3-NT suggests increased interaction of superoxide and nitric oxide to create nitrotyrosine (nitration of tyrosine residues mainly within proteins). Moreover,

total antioxidant status (TAS), which is a combined measurement of systemic antioxidant defence capacity, evaluates the interaction between oxidant generation and the effectiveness of the antioxidant buffering system[9,10]. The objective of this study was to investigate the comparison between allopurinol and febuxostat on oxidative and nitrosative stress markers in a doxorubicin-induced oxidative stress model in rats. We examined the levels of serum NO, TAS, MDA, and 3-NT to determine if differences in antioxidant protection and nitrosative damage were responsible for the varied biological effects of these two XO inhibitors.

MATERIAL AND METHODS

Experimental design: Forty healthy male albino rats weighing 230–350 g were used in this study. The animals were obtained from the animal house of the College of Veterinary Medicine, University of Mosul. The animals were housed under standard laboratory conditions: temperature (22–25°C) and a 12-hour light/dark cycle. The animals were acclimatized for one week before initiation of the experiment to minimize stress-related physiological variations.

Ethical Approval: The study protocol was reviewed and approved by the Scientific and Ethical Committee of the College of Medicine, University of Mosul. (Ref. no. UOM/COM/MREC/25-26/APR10/B, Date 27/4/2026.)

Rats were randomly allocated into four experimental groups (10 rats per group): control group (C), doxorubicin-administered-only-treated group (D), allopurinol-plus-doxorubicin-treated group (A), and febuxostat-plus-doxorubicin-treated group (F). The sample size was selected based on previous experimental studies evaluating doxorubicin-induced cardiovascular injury and the protective effects of xanthine oxidase inhibitors in rats. This sample size has been consistently shown to provide adequate statistical power to detect biologically relevant differences in biochemical, vascular, and histopathological outcomes while adhering to principles of reduction in animal research. Two weeks for induction of oxidative and nitrosative stress by doxorubicin. Concomitant treatment with allopurinol and febuxostat was done with doxorubicin (total 3 weeks) to protect against doxorubicin-induced oxidative injury. Doxorubicin was administered intraperitoneally at a dose of 2.5 mg/kg on days 2, 4, 6, 9, and 14 of the experiment, for a cumulative dose of 12.5 mg/kg. This repeated-dose protocol was selected to induce progressive oxidative stress and cardiovascular injury while maintaining acceptable animal survival. Allopurinol was taken orally once daily at the experimental dosage. The medication was newly produced for each delivery and given at 50 mg/kg/day. Febuxostat was delivered orally once daily during the research period at the indicated experimental dosage. The medication was made afresh before each dose and given at 10 mg/kg/day[11]. The doses of doxorubicin, allopurinol, and febuxostat were selected based on previously validated experimental studies. Doxorubicin was administered intraperitoneally at a cumulative dose of 12.5 mg/kg over 21 days to induce reproducible cardiovascular injury while minimizing excessive mortality. Allopurinol (50 mg/kg/day) and febuxostat (10 mg/kg/day) were administered orally throughout the experimental period because these doses have been shown to produce effective xanthine oxidase inhibition and exert antioxidant and cardioprotective effects in rat models of oxidative stress and doxorubicin-induced cardiotoxicity[12]. The selected regimens allowed comparison of the protective effects of both xanthine oxidase inhibitors against cumulative doxorubicin-induced cardiovascular injury[13].

Blood was collected from the retro-orbital venous plexus both at baseline and post-treatment[14]. From each animal, 5 mL of venous blood was drawn and centrifuged to separate serum. The serum was stored at −20°C until the biochemical analysis was done. A commercially available ELISA kit assessed serum NO, 3-NT, MDA, and TAS levels according to the manufacturer's recommendations.

Determination of nitric oxide (NO): Serum nitric oxide levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. Absorbance was measured spectrophotometrically, and NO concentrations were calculated using the standard curve provided with the kit.

Determination of 3-Nitrotyrosine (3-NP): Serum 3-Nitrotyrosine concentrations were quantified using a rat-specific ELISA kit according to the manufacturer's protocol. The assay is based on antibody-mediated detection of nitrated proteins, and absorbance values were measured using a microplate reader.

Determination of Total Antioxidant Status (TAS): Total antioxidant status was measured using a colorimetric ELISA-based assay according to the manufacturer's instructions. TAS values represent the cumulative antioxidant capacity of serum samples against reactive oxygen species.

Determination of Malondialdehyde (MDA): Serum malondialdehyde was analyzed by an ELISA kit based on antibody-mediated detection of MDA.

Histopathological Examination: At the end of the experimental study, the aortic tissues were collected for histopathological examination. The tissues were fixed in 10% formalin solution, processed routinely, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Histopathological examination was performed under light microscopy to evaluate myocardial degeneration, inflammatory infiltration, vascular congestion, endothelial injury, smooth muscle degeneration, necrosis, and structural alterations in cardiac and aortic tissues.

Statistical analysis: Data were analyzed using GraphPad Prism version 10.0 (USA). Results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc test was used to compare studied groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Malondialdehyde (MDA): The determination of serum MDA levels indicated a highly significant increase ($p<0.001$) in the doxorubicin-treated group D compared to control group C, reflecting increased lipid peroxidation and oxidative membrane damage. Both allopurinol (A) and febuxostat (F) treatment significantly decreased MDA concentrations as compared to the D group, bringing values closer to normal levels. Both treatment groups had similar outcomes (Figure 1).

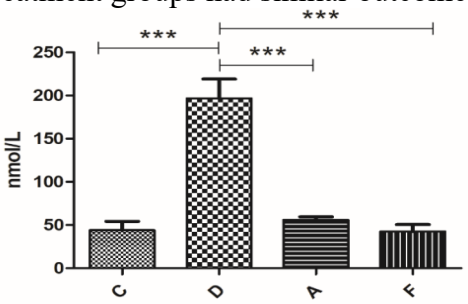

Figure (1): Effect of allopurinol and febuxostat on serum malondialdehyde (MDA) level in rats treated with doxorubicin relative to control (C). The D group significantly increased MDA concentrations compared with the control; (A) and (F) groups significantly reduced MDA concentrations relative to **(D)**. (***P<0.001). No significant difference was observed between (A)- and (F)-treated groups. Data are presented as mean ± SD.

Total antioxidant status (TAS): TAS levels in the doxorubicin-treated group were markedly higher compared to controls. While numerical increases in TAS values were noted between allopurinol- and febuxostat-treated groups, especially in the F group, the differences did not reach statistical significance compared with that of the D group (Figure 2).

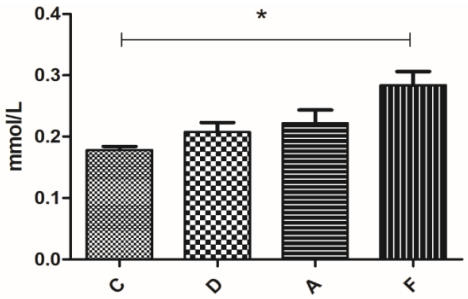

Figure (2): Effect of allopurinol and febuxostat on total antioxidant status (TAS) in rats treated with doxorubicin relative to control (C). (D) increased TAS concentrations compared with control and (F) significantly increased TAS concentrations relative to (C). (*P<0.05). No significant difference was observed between A- and F-treated groups. Data are presented as mean±SD.

Nitric oxide (NO): The D group had higher serum NO concentrations than controls. After treatment, numerical changes were noticed between groups, with lower values in the allopurinol group and slightly higher values in febuxostat compared to D, but differences did not reach statistical significance compared with C; (A) was significantly lower than (D) and (F) (Figure 3).

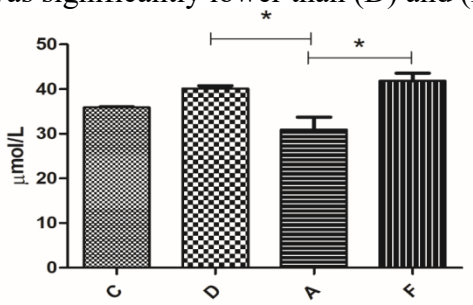

Figure 3. Effect of allopurinol and febuxostat on nitric oxide concentrations (NO) in rats treated with doxorubicin relative to control (C). No differences were observed in the level of NO in (D) compared to control; however, (A) significantly reduced NO relative to (D) and (F) groups. (*P<0.05). Data are presented as mean ± SD.

3-Nitrotyrosine (3-NT): The serum 3-NT concentrations were significantly higher in the D, A, and F groups than in controls. Allopurinol treatment showed a small numerical decrease, but febuxostat values were similar to or slightly increased compared to the values determined in the D group. However, neither treatment caused a significant change in 3-NT concentrations compared with (D) (Figure 4).

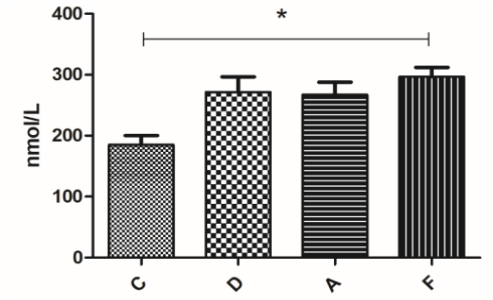

Figure 4. Effect of allopurinol and febuxostat on 3-nitrotyrosine (3-NT) in rats treated with doxorubicin relative to control (C). (D) and (A) slightly increased the concentration of 3-NT relative to control; however, there was a significant increase in the level of 3-NT in the F-treated group (*P < 0.05). Data are presented as mean±SD.

Histology analysis

The control group (Figure 5C) exhibited the normal histological architecture of the aortic wall, characterized by an intact tunica intima with a continuous endothelial lining, a well-organized tunica media composed of regular smooth muscle fibers, and an unremarkable tunica adventitia. In contrast, the doxorubicin-treated group (Figure 5D) demonstrated severe vascular injury, including the presence of numerous scattered foam cells, marked thickening and disorganization of the smooth muscle fibers, and the formation of cholesterol clefts within the tunica media, indicative of significant atheromatous change. The administration of allopurinol alongside doxorubicin (Figure 5A) resulted in a partial amelioration of these effects, as evidenced by only mild foam cell infiltration in the tunica media and the preservation of smooth muscle fiber integrity; however, some inflammatory cell infiltration persisted in the tunica adventitia. Notably, the combination of febuxostat with doxorubicin (Figure 5F) afforded the most pronounced protective effect, yielding a near-normal aortic wall architecture. This group showed an intact tunica intima and endothelium, well-preserved smooth muscle fibers in the tunica media, with only a single mild foam cell and minimal inflammatory cell infiltration in the adventitia.

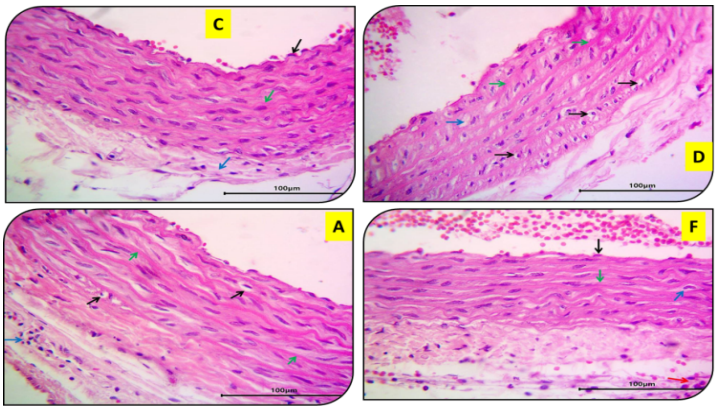

Figure 5. A representative histological section of rat aorta from the studied groups. **(C)** Control group showing intact architecture of the tunica intima with the endothelium [→], tunica media with the smooth muscle cells [→], and tunica adventitia [→]. **(D)** Histological section of rat aorta from the Doxorubicin group showing high scattered foam cells [→], thickening of the smooth muscle fibers [→], and cholesterol clefts in the tunica media [→]. **(A)** Histological section of rat aorta from the allopurinol and Doxorubicin group showing mild scattered foam cells in the tunica media [→] with intact smooth muscle fibers [→], and inflammatory cells infiltrate the tunica adventitia [→]. **(F)** Histological section of rat aorta from the febuxostat and doxorubicin treated group showing intact architecture of the tunica intima with the endothelium [→] and intact tunica media with smooth muscle fibers [→], with a single mild presence of foam cells [→] and inflammatory cell infiltration in the tunica adventitia [→]. H&E stain, 400X.

DISCUSSION

In this study, we have observed that doxorubicin induces a clear oxidative and nitrosative damage profile in rats, indicated by markedly increased serum levels of MDA, NO, 3-NT, and TAS when compared to the control group. This observation is consistent with the previously established mechanism of doxorubicin-induced toxicity in which redox cycling of anthracyclines and thiol modification leads to an overproduction of reactive oxygen species (including superoxide and hydrogen peroxide), disruption of mitochondrial homeostasis, lipid peroxidation and inflammation, nitric oxide dysregulation, and peroxynitrite formation. Recent reviews show that oxidative stress, nitrosative stress, inflammation, apoptosis, ferroptosis, and mitochondrial injury are the neurodegeneration-critical mechanisms of doxorubicin toxicity[13,15].

The MDA content in the doxorubicin-treated group was significantly elevated, suggesting oxidative membrane damage via greater lipid peroxidation. This outcome aligns with other studies in showing that doxorubicin elevated lipid peroxidation mediated by ROS targeting polyunsaturated fatty acids within the cellular and mitochondrial membranes. Allopurinol and febuxostat significantly reduced MDA concentrations to levels lower than those of the doxorubicin-treated group,

indicating that they are good protectors against lipid peroxidation. Inhibition of xanthine oxidase-derived reactive oxygen species is probably the most likely mechanism. Lowering the production of superoxide and hydrogen peroxide reduces the initiation of lipid peroxidation and restricts the propagation of oxidative chain reactions in biological membranes. This is consistent with the results of previous studies that discovered that inhibiting xanthine oxidase could reduce XO-derived oxidative stress and/or cell death signalling, preventing doxorubicin cardiotoxicity[16]. A decrease in lipid peroxidation may also preserve mitochondrial integrity. Because excessive xanthine oxidase-derived ROS can further increase mitochondrial oxidative stress, inhibition of xanthine oxidase may break this feed-forward cycle by dampening electron leakage and normalizing oxidative phosphorylation to reduce subsequent ROS generation. Based on the importance of mitochondrial permeability transition pores in lipid peroxidation, preservation of more intact mitochondrial membranes may thus play a large role in reducing both intracellular and systemic MDA levels. While MDA reduction from both drugs was similar, this assay may not fully represent the mechanism of action for either drug. Allopurinol and its active metabolite oxypurinol have been found to demonstrate antioxidant activities beyond pure xanthine oxidase inhibition, including direct free radical scavenging and restoration of purine salvage pathways, thus preserving intracellular ATP stores. In contrast, Febuxostat is a nonpurine, selective, and more potent xanthine oxidase inhibitor with considerable inhibition of both the oxidized and reduced forms. Therapeutically, this property led to greater suppression of xanthine oxidase-derived ROS by febuxostat, while allopurinol produced wider-spread but non-specific antioxidant effects[17,18].

One of the interesting findings in this study was that the plasma total antioxidant status level was significantly increased in the group with doxorubicin treatment. At first sight, this might seem paradoxical because oxidative stress is mostly associated with depletion of antioxidants. Increasing TAS is not always an indicator of enhanced antioxidant defence. Rather, it might. When ROS production is in excess, endogenous antioxidant systems are activated to recover redox homeostasis. Increased synthesis or mobilization of uric acid, bilirubin, albumin, glutathione, and antioxidant enzymes may transiently raise circulating antioxidant capacity while organs remain in an oxidative state. Thus, the higher TAS after exposure to doxorubicin likely measures compensatory defence mechanisms and not protection against oxidative damage. Even though lipid peroxidation was significantly reduced with allopurinol and febuxostat, normalization of TAS values was not significant[19,20]. This is relevant as it argues that xanthine oxidase inhibition diminished oxidative injury without normalizing systemic redox status. The continued generation of widespread mitochondrial ROS via xanthine oxidase, persistent inflammatory activation, and increased NADPH oxidase activity and inducible nitric oxide synthase activation may contribute to oxidative stress[21]. The increased tendency toward higher TAS values observed in the febuxostat-treated animals may also be a sign of persistent adaptation, activating endogenous antioxidant systems or other molecules that exert adaptive actions. Because physiological levels of reactive oxygen species act inside cells as messengers, regulating cellular signalling pathways that control antioxidant gene expression through transcription factors such as Nrf2, excessive suppression of ROS may disturb normal redox signalling and slow recovery to a state of physiological antioxidant homeostasis. This notion of redox modulation rather than complete ROS elimination has drawn increasing interest for cardiovascular effects and might help explain the observed differences between selective and non-selective xanthine oxidase inhibition in animal or human trials[22].

Nitric oxide is a major regulator of both vascular homeostasis and endothelial function. At physiological levels, endothelium-derived nitric oxide (NO) expressed from endothelial nitric oxide synthase (eNOS) regulates vascular relaxation and inhibits platelet aggregation and leukocyte adhesion by inhibiting the expression of adhesion molecules as well as avoiding vascular smooth muscle proliferation. Nonetheless, under inflammatory and oxidative states, nitric oxide biochemistry is strongly modified. Thus, the pronounced rise of serum nitric oxide in the doxorubicin group will mostly reflect pathological nitric oxide generation rather than reduced endothelial function[23,24]. This finding can be explained by several different mechanisms. One of them is oxidative stress induced by doxorubicin, which activates inflammatory transcription factors, particularly NF-κB, leading to elevated expression of iNOS. In contrast with eNOS, which produces low physiological amounts of NO continuously, iNOS can produce large quantities of NO during periods. Thus, serum NO levels increase in parallel with impaired endothelial function. Hence, raised circulating nitric oxide levels may always be viewed as an indicator of inflammatory activation rather

than maintenance of endothelial integrity[25]. Administration of allopurinol produced a significant reduction in serum nitric oxide concentrations compared with the doxorubicin-treated 50 group, whereas febuxostat failed to produce a comparable reduction and maintained nitric oxide levels close to those observed in the doxorubicin group. These findings suggest that although inhibition of xanthine oxidase effectively reduces one important source of reactive oxygen species, inflammatory nitric oxide production, primarily mediated by inducible nitric oxide synthase (iNOS), may persist despite treatment. Thus, suppression of oxidative stress alone may not be sufficient to fully normalise nitric oxide metabolism in doxorubicin-induced vascular injury[24,26]. Compared with febuxostat, allopurinol significantly lowered nitric oxide concentrations. This difference may reflect antioxidant actions extending beyond xanthine oxidase inhibition. The active metabolite of allopurinol, oxypurinol, has been shown to directly scavenge reactive oxygen species and attenuate inflammatory signalling, thereby reducing iNOS activation and limiting excessive nitric oxide production. In contrast, the more selective inhibition of xanthine oxidase by febuxostat may have less influence on inflammatory pathways responsible for nitric oxide overproduction, which could explain the persistently elevated nitric oxide concentrations observed in the febuxostat-treated group[27].

Doxorubicin treatment significantly increased 3-NT, with evidence of nitrosative stress and peroxynitrite-mediated protein nitration[28]. This is expected since superoxide spontaneously reacts with NO to yield peroxynitrite, which nitrates tyrosine residues and produces 3-NT. Allopurinol caused a small decrease in 3-NT, febuxostat had values comparable to or slightly higher than doxorubicin alone, and none of these was statistically significant. It implies that XO inhibition decreased lipid peroxidation; however, it failed to effectively inhibit the NO–superoxide–peroxynitrite axis. The more favorable numerical pattern seen with allopurinol could be due to its wider antioxidant activity in addition to XO inhibition, while febuxostat's stronger and more selective XO inhibition may not provide the same level of protection against protein nitration[29].

Overall, the study suggests both oxidative and nitrosative injury from doxorubicin. Allopurinol and febuxostat significantly inhibited MDA, a marker of lipid peroxidation, compared with the control ($P<0.001$), but the inhibition effect on TAS and 3-NT was not statistically significant. Thus, the major confirmed protective effect of both drugs is anti-lipid-peroxidative rather than complete antioxidant or antinitrosative restoration. In accordance with the widely recognized concept of redox modulation, allopurinol seemed to numerically exert a better effect on NO, although febuxostat achieved significant reductions of MDA parallel to effective control of nitrosative stress as an independent mechanism. These findings may explain why the cardiovascular safety of febuxostat is still debated clinically, as CARES showed an increase in cardiovascular mortality and FAST found that febuxostat was non-inferior to allopurinol in different patients[30,31].

The histopathological findings have given direct morphological evidence for biochemical and functional changes associated with doxorubicin administration. The marked structural injury in aortic tissues, equivalent to significant increases in serum MDA, NO, and 3-nitrotyrosine, was consistent with oxidative/nitrosative stress leading to substantial tissue damage[32].

Histological assessment of the control tissue showed regular vascular structure. Doxorubicin administration caused the aorta to exhibit plaque wall thickening, foam cell accumulation, and lipid migration, leading to smooth muscle proliferation, the presence of cholesterol clefts, and infiltration of inflammatory cells[33]. These changes in structure support the significant increase in MDA determined in this study, which correlates with increased lipid peroxidation and membrane damage[34]. Allopurinol treatment markedly reduced these pathological changes. On histology, only mild hyaline degeneration, edema, and vascular congestion were present, while the aortic architecture was mostly preserved. Interestingly, febuxostat had a better sparing effect on vascular architecture compared to allopurinol, although there were a few biochemical circulating markers such as NO and 3-nitrotyrosine, which improved slightly more with allopurinol. Febuxostat treatment also preserved more endothelial-derived lining of vascular smooth muscle, consistent with the ability of febuxostat to decrease vascular oxidative stress and improve endothelial function[35].

CONCLUSION

The biochemical and histopathological findings indicate that oxidative stress plays a central role in doxorubicin-induced oxidative stress. Inhibition of xanthine oxidase effectively reduces lipid peroxidation and partially restores vascular function, confirming the contribution of xanthine oxidase-derived ROS to the pathogenesis of anthracycline-induced vascular toxicity. However, the persistence of elevated 3-nitrotyrosine levels and the incomplete restoration of total antioxidant status (TAS), despite the significant reduction in nitric oxide by allopurinol, suggest that multiple interconnected redox pathways participate in the development of cardiovascular injury and should be considered when designing future therapeutic strategies. Overall, this study provides experimental evidence supporting the protective effects of xanthine oxidase inhibition against doxorubicin-induced vascular injury.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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