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Cardioprotective Impact of Aqueous *Saussurea lappa* Root Extract in Female Rats Treated with Tamoxifen

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Abstract:
Tamoxifen (TMX), a widely used anti-estrogenic agent for breast and ovarian cancer therapy, has been associated with cardiotoxicity upon long-term administration. *Saussurea lappa* (Indian Costus root) possesses potent antioxidant and cardioprotective properties in preclinical studies, suggesting its potential in preventing drug-induced cardiac injury. This study investigated the cardioprotective efficacy of *Saussurea lappa* root extract (SLRE) against TMX-induced cardiotoxicity in vivo. Experimental animals were divided into four groups: control, SLRE-treated (200 mg/kg), TMX-treated (40 mg/kg), and a combination group receiving both agents. All treatments were administered orally over 28 days. Cardiac injury was assessed by measuring serum biomarkers lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), and troponin I and by histopathological evaluation of myocardial tissue. TMX administration led to significant elevations in cardiac biomarkers and notable histopathological damage, indicative of cardiotoxicity. Co-administration of SLRE markedly reduced LDH, CK-MB, and troponin I levels ($P < 0.001$) and preserved cardiac tissue architecture. These findings suggest that SLRE mitigates TMX-induced cardiac injury, likely through antioxidant and anti-inflammatory mechanisms. The study highlights the therapeutic potential of SLRE as a natural cardioprotective agent and encourages further investigation into its mechanisms and clinical applications.

Keywords: Cardiotoxicity, Saussurea lappa extract, Female rats, Tamoxifen

التأثيرات الوقائية للمستخلص المائي لجذور نبات القسط الهندي (*Saussurea lappa*) ضد السمية القلبية الناتجة عن التاموكسيفين في إناث الجرذان

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الملخص
يُعدّ التاموكسيفين عاملًا مضادًا للإستروجين يُستخدم على نطاق واسع في علاج سرطاني الثدي والمبيض، وقد ارتبط استخدامه المطوّل بظهور سمّية قلبية، رغم عدم وضوح الآليات المسؤولة عن هذه التأثيرات. في المقابل، أظهر نبات القسط الهندي (*Saussurea lappa*) خصائص قوية مضادّة للأكسدة وواقية للقلب في النماذج ما قبل السريرية، ممّا يشير إلى دوره المحتمل في التخفيف من التلف القلبي الناجم عن الأدوية. هدفت هذه الدراسة إلى تقييم التأثير الوقائي لمستخلص جذور القسط الهندي (SLRE) ضد السمية القلبية الناتجة عن التاموكسيفين، وذلك من خلال تحليل المؤشرات الحيوية في مصل الدم(LDH) ، CK-MB، والتروبونين I ، إلى جانب الفحص النسيجي لعضلة القلب. تم توزيع الحيوانات التجريبية على أربع مجموعات: مجموعة ضابطة، مجموعة تلقت المستخلص المائي (SLRE) بجرعة 200 ملغم/كغم، مجموعة تلقت التاموكسيفين بجرعة 40 ملغم/كغم، ومجموعة تلقت العلاجين معًا. استمر الإعطاء الفموي لمدة 28 يومًا. أظهرت مجموعة التاموكسيفين ارتفاعًا معنويًا في مؤشرات الدم وتغيرات نسيجية كبيرة، مما يعكس آثارًا سمّية واضحة على القلب. بالمقابل، أدى العلاج المشترك مع SLRE إلى انخفاض معنوي في المؤشرات القلبية($P < 0.001$) ، وتحسن واضح في البنية النسيجية لعضلة القلب وانخفاض في الارتشاح الالتهابي. تشير النتائج إلى أن لمستخلص القسط الهندي تأثيرًا وقائيًا فعّالًا ضد السمية القلبية الناتجة عن التاموكسيفين، ويُرجّح أن تعزى هذه الحماية إلى خواصه المضادّة للأكسدة والالتهاب. وتوصى بإجراء دراسات آلية وتجارب سريرية إضافية لتأكيد هذه النتائج واستكشاف إمكاناته العلاجية المستقبلية.

الكلمات المفتاحية: السمية القلبية، مستخلص القسط الهندي، إناث الجرذان، التاموكسيفين.

Introduction

With continuous advancements in antitumor therapies, survival rates among cancer patients have markedly increased. However, this progress has been accompanied by a rise in treatment-associated complications. Among these, cardiotoxicity induced by antitumor agents has become a significant clinical challenge, forcing clinicians to balance effective oncologic management with the preservation of cardiac function. This dilemma is further exacerbated by overlapping risk factors such as genetic predisposition, smoking, and obesity that contribute to both cancer and cardiovascular diseases. Moreover, molecular signaling pathways that regulate cardiovascular homeostasis are intricately involved in tumorigenesis, progression, and metastasis, highlighting a complex interrelationship between cancer and cardiovascular health [1]. Consequently, antitumor treatments may disrupt cardiovascular equilibrium, increasing the risk of cardiovascular disorders [1]. Tamoxifen (TMX), a widely used drug in breast cancer therapy, has been reported to improve triglyceride profiles in patients [2]. Nevertheless, its administration is linked to a higher incidence of venous thromboembolism and stroke when compared to controls [3]. Furthermore, TMX exerts notable effects on cardiac electrophysiology, with evidence indicating a stronger association than aromatase inhibitors with drug-induced long QT syndrome, torsade de pointes ventricular tachycardia, and other ventricular arrhythmias [4]. Observational clinical studies have revealed that TMX use correlates with elevated risks of myocardial infarction, ischemic stroke, and heart failure, with cardiovascular events attributable to TMX estimated at approximately 2% [5]. Despite these clinical observations, the precise mechanisms underlying TMX-induced cardiotoxicity remain poorly defined. Some research suggests that upregulation of estrogen receptor expression may inhibit pro-inflammatory mediators such as MCP-1, TNF-α, and IL-6, thereby exerting anti-inflammatory effects [6, 7]. Estrogen receptors perform multifaceted cardioprotective roles, including anti-apoptotic, pro-hypertrophic, anti-inflammatory, anti-atherosclerotic, vasodilatory, and angiogenic effects [8]. Supporting this, Meng et al. reported that estrogen facilitates atherosclerosis prevention by promoting autophagy and reducing endothelial apoptosis [9]. These findings have prompted the hypothesis that TMX-induced myocardial injury might result from suppression of estrogen receptor activity.In recent years, accumulating evidence has underscored the crucial role of oxidative stress imbalance in the pathogenesis of myocardial injury. Excessive generation of superoxide anions and reactive oxygen species (ROS) substantially contributes to organelle dysfunction and cellular damage [10]. Estrogen and its receptors are believed to mitigate oxidative stress via dual mechanisms: suppression of ROS production and enhancement of ROS clearance in both myocardial and vascular tissues [11]. These insights underscore the importance of early detection, prevention, and treatment of cardiac injury to improve patient outcomes. Natural antioxidants are widely recognized for their ability to alleviate oxidative stress primarily through scavenging free radicals [12]. Saussurea lappa, a medicinal plant abundant in antioxidant compounds, has a longstanding history in traditional medicine. Known as "Kushta" in Sanskrit, S. lappa Clarke (family Compositae) is a tall perennial herb native to the Kashmir region. Its hot water root extract has traditionally been employed in managing asthma, inflammation, and rheumatism [13]. The roots are characterized by their hot, bitter, mildly sweet, pungent, and carminative properties, and have been used as analgesics, digestives, aphrodisiacs, and diuretics. Numerous studies have documented diverse pharmacological effects of S. lappa roots, including cortisol-lowering, bronchodilatory, anti-ulcer, anticancer, anti-inflammatory, antiviral, and hepatoprotective actions [14]. Traditionally, the aqueous root extract has also demonstrated anti-anginal activity [15]. Building on these attributes, the present study aims to evaluate the cardioprotective efficacy of the aqueous extract of S. lappa (AESL) roots against TMX-induced cardiac toxicity in rats, thereby providing scientific validation for its traditional use in mitigating Tamoxifen-related cardiotoxicity.

Methods

Chemicals

Tamoxifen (tamoxifen citrate), commercially available as Nolvadex® and supplied by AstraZeneca, United Kingdom, was used in this study in tablet form. For experimental administration, the tablets were suspended in distilled water and given orally to the animals at a dose of 40 mg/kg body weight. This dose corresponds to the human therapeutic equivalent and was administered once daily for 28 consecutive days, following the protocol established by Paget and Barnes [16].

Aqueous Extraction Protocol for *S. lappa* Root

Dried roots of *S. lappa* were procured from a medicinal plant market in Benghazi, Libya. For the preparation of the aqueous extract, 1 kg of the powdered root was boiled in 5 liters of distilled water for 30 minutes, followed by filtration. The resulting solution was subsequently lyophilized to obtain a freeze-dried extract. For use in the current study, the lyophilized material (approximately 35 g) was reconstituted in distilled water to achieve a final concentration of 50 mg/ml [17].

Study design and setting

A total of twenty-four female albino rats, aged eight weeks and weighing between 180 and 190 grams, were utilized in this study. The animals were procured from the Animal House at the Faculty of Medicine, University of Benghazi. They were maintained under standard laboratory conditions, including a temperature range of 23–25 °C, relative humidity of 50–65%, and a 12-hour light/dark cycle. The rats had free access to a standard pellet diet and water throughout the study period.

Following a seven-day acclimatization period, the animals were randomly assigned into four groups, each comprising six rats:

Group I (Control): Received oral administration of normal saline and served as the untreated control.

Group II (SLRE): Administered orally with *Saussurea lappa* root extract (SLRE) at 200 mg/kg/day for 28 consecutive days [17].

Group III (TMX): Treated orally with tamoxifen suspension at 40 mg/kg/day for 28 consecutive days [16].

Group IV (TMX + SLRE): Received concurrent oral administration of tamoxifen (40 mg/kg/day) and SLRE (200 mg/kg/day) for 28 consecutive days.

Data collection procedure

Blood Collection and Serum Biochemical Analysis

At the conclusion of the experimental period, the rats were euthanized by cervical dislocation. Blood samples were obtained through cardiac puncture using heparinized tubes and subsequently centrifuged at 3000–4000 rpm for 10 minutes to separate the serum. Serum concentrations of cardiac injury biomarkers including creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and cardiac troponin I (cTnI) were quantified following the standard protocols associated with the commercially available diagnostic kits.

Histological Examination of Cardiac Tissue

Immediately after sacrifice, hearts were excised, rinsed with ice-cold saline, and fixed in 10% buffered formalin. Following fixation, tissues were processed, embedded in paraffin, and sectioned at 5 µm thickness using a microtome. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope to evaluate structural changes, including myocardial degeneration, inflammatory cell infiltration, and overall tissue integrity [18].

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) performed with SPSS software version 27 (SPSS Inc., Chicago, IL, USA). Results are presented as mean ± standard deviation (SD). Post hoc multiple comparisons between groups were conducted using Tukey's test. Statistical significance was set at a p-value less than 0.05.

Results

Biochemical results

Serum LDH

The impact of *Saussurea lappa* root extract on serum LDH activity as a biomarker of heart tissue damage in different experimental groups is shown in **figure 1**. As shown in the figure , non significant change in LDH activity in rats treated with S. *lappa* extract only when compared with control ones , while significant elevation in this cardia damage index in serum of TMX intoxicated rats in comparison to control animals (P=0.001). Co-intake of SLRE with TMX markedly ameliorated serum LDH activity in TMX- SLRE treated group with respect to TMX intoxicated group (P=0.001).

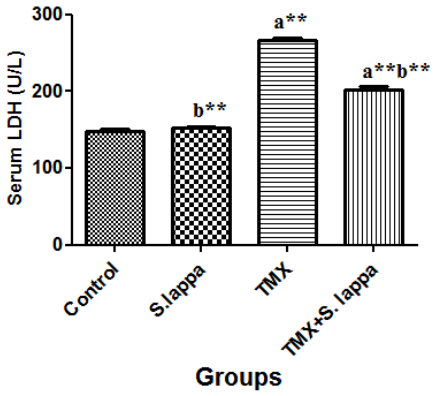

Figure1. Effect of SLRE (*S. lappa*) on serum LDH activity in TMX induced cardiac damage in rats. Values are expressed as mean ± SD of 6 rats, a***P*=0.001 compared control, ^{b**}*P*= 0.001 compared with TMX

Serum CK-MB

The impact of SLRE on serum CK-MB activity in different experimental rat groups as another marker of cardiac dysfunction is depicted in **Figure 2**. The data demonstrated non significant change in CK-MB activity in rats treated with SLRE only when compared with control animals , while significant elevation in this cardia damage index in serum of TMX intoxicated rats in comparison to control animals (*P*=0.001). Co-intake of SLRE with TMX greatly down-modulated serum CK-MB activity in TMX- SLRE treated group relative to TMX intoxicated group (*P*=0.001).

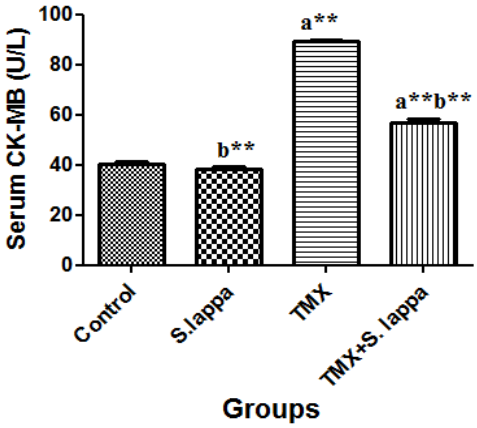

Figure2. Effect of SLRE (*S. lappa*) on serum CK-MB activity in TMX induced cardiac damage in rats. Values are expressed as mean ± SD of 6 rats, a***P*=0.001 compared control, ^{b**}*P*= 0.001 compared with TMX

Serum Troponin I

Figure3. The results demonstrate the effect of Saussurea lappa root extract (SLRE) on serum troponin I levels, a key biomarker of cardiac tissue injury, across control and tamoxifen (TMX)-treated groups. No significant difference in troponin I levels was observed in rats treated with SLRE alone compared to the control group. In contrast, TMX-intoxicated rats exhibited a significant elevation in serum troponin I levels relative to controls (P = 0.001). Notably, co-administration of SLRE with TMX significantly reduced serum troponin I concentrations in the TMX + SLRE group compared to the TMX-only group (P = 0.001).

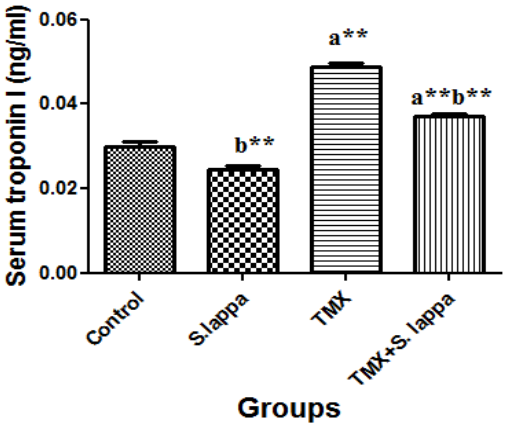

Figure 3. Impact of SLRE (*S. lappa*) on serum troponin I concentration in TMX induced cardiac damage in rats. Values are expressed as mean ± SD of 6 rats, a$^{**}P$=0.001 compared control, $^{b**}P$= 0.001 compared with TMX

Histopathological result

As illustrated in **Figure 4**, histolmorphological pictures of cardiac sections of control rats and rats treated with SLRE only **(Figure 4A & B respectively)** revealed normal cardiac tissue architecture with normal myocytes. Histologic section of cardiac section from rat treated with TMX showed myocardial fiber fracture in some areas and some myocytes with pyknotic nuclei and others with Karyolysis nuclei **(Figure 4C)**. Cardiac section from rat treated with TMX along with SLRE showed more or less normal cardiac architecture **(Figure 4D)**

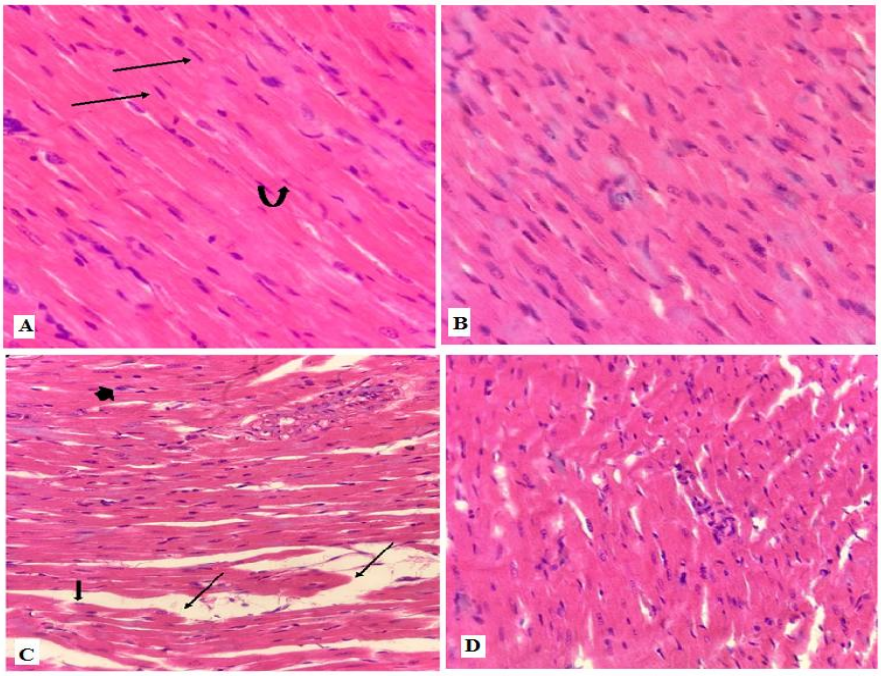

Fig4. Effect of SLRE (*S. lappa*) **on** histological pictures of cardiac tissue in TMX induced

cardiotoxicity in rats (A) Normal control cardiac rat tissue showing normal myocytes with normal nuclei (arrows) and normal intercalated disc (curved arrow). (B) Picture of cardiac section from rat treated with SLRE showing normal cardiac architecture. (C) Picture of cardiac section from rat treated with TMX showing myocardial fiber fracture (thin arrows), some myocytes with pyknotic nuclei (arrow head) and others with Karyolysis nuclei (small arrow) (D) Picture of cardiac section from rat treated with TMX along with SLRE showing more or less normal cardiac architecture (H&E, x400).

Discussion

moxifen (TMX) has been extensively used in clinical practice for many years [19], and its cardiotoxic effects have been documented in numerous studies [1,2]. Nevertheless, TMX-induced myocardial injury remains an

unresolved clinical concern, posing a significant risk of cardiovascular events among cancer survivors. So searching for an agent to prevent or reduce the cardiotoxic impact of TMX is becoming a crucial requirement for subjects with breast cancer. In the current study, the prophylactic effect of SLRE against TMX induced cardiac injury in female rats was investigated. The results of the current work revealed that administration of TMX induced cardiac tissue damage as shown by significant increases in serum cardiac enzymes,namely LDH and CK-MB as well as cardiac protein namely troponin I (biomarkers of myocyte injury) in rats. Intoxicated with TMX relative to TMX untreated intoxicated ones. The damaging impact of TMX was further confirmed by severe disorganization of histo -morphological pictures of cardiac tissue as observed by myocardial fiber fracture in some areas and some myocytes with pyknotic nuclei and others with Karyolysis nuclei. The current biochemical results as well as the histopatholgical observation are coped with previous investigation revealed that alteration in cardiac function markers and cardiac histomorphological picture of tumor-bearing mice in response to TMX treatment, suggesting that the loss of cardiomyocyte was a main cause of TMX-related myocardial injury in TMX-treated mice [4]. Cardiac LDH, CK-MB and troponin I are sensitive and precise indicators of myocardial damage [20]. The significance of this cytosolic pool lies in its role as the source of troponins released into circulation within 4–6 hours following myocardial injury. The continued degradation of myofibrils in injured cardiomyocytes contributes to the sustained elevation of troponin I levels in the bloodstream [20]. Both troponin I and CK-MB are released from damaged cardiac tissue and are widely utilized as diagnostic biomarkers for predicting serious cardiovascular events, including myocardial infarction and mortality [21].The damaging effect of TMX on cardiac tissue may relate to the ability of TMX to induce oxidative stress in cardiac muscle fibber, leading destruction of myocytes. This can, result in enzymatic leakage into circulation [4]. Also, Meng et al [4] reported that the changes in heart function may relate to the ability of TMX to induce cardiac apoptotic cell death via the IL-6/p-STAT3/PGC-1α/ROS axis, causing cardiac dysfunction. In addition, the same authors stated that TMX promotes the release of inflammatory cytokines which cause tissue necrosis, resulting in further damage to myocytes [4]. Protective oral administration of SLRE significantly supressed the elevation of cardiac damage indices (LDH, CK-MB and troponin I), proposing the potential cardioprotective impact of SLRE. The plant extract also greatly protected the cardiac architecture from damaging influence of TMX as observed by normal heart architecture with normal nucleus. The prophylactic beneficial impact of SLRE. May correlate to its phytochemical constituents. *S lappa* plant contains phenolic acids and flavonoids with antioxidant properties. These compounds can prevent oxidative tissue damage and preserve membrane integrity [22].

Conclusion

The findings of the present study suggest that *Saussurea lappa* root extract (SLRE) exhibits significant cardioprotective effects against tamoxifen (TMX)-induced myocardial injury. This protective action may be attributed to its antioxidant, anti-inflammatory, and oxidative stress-reducing properties. These results highlight the potential therapeutic value of SLRE in mitigating TMX-associated cardiac damage in breast cancer patients.

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