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Nephroprotective effects of *Saussurea lappa* root aqueous extract against tamoxifen-induced renal damage in female rats

Eman Ali Faraj Hamouda

Department of Zoology, Factually of Arts and Sciences, University of Benghazi, Tocra, Libya

eali40411@gmail.com

الملخص:

يُعتبر سرطان الثدي أكثر الأورام شيوعًا الذي يؤثر بشكل رئيسي على الأفراد من الجنس الأنثوي على مستوى العالم، يستخدم عقار التاموكسيفين (TAM) بكثرة لعلاج هذا النوع من السرطان، ومع ذلك أظهرت الأبحاث أن الاستخدام المطول لهذا العقارقد يؤدي إلى السمية الكلوية.

تهدف الدراسة الي تقييم التأثير الوقائي للمستخلص المائي لجذور نبات القسط الهندي (AESL) لتخفيف الضرر الكلوي الناتج عن المعالجة بعقار التاموكسيفين .(TAM). أجريت الدراسة علي عدد (24) من إناث الجرذان بوزن يتراوح مابين 180–190 جرام ، تم تقسيم الجرذان بشكل رئيسي إلى أربع مجموعات: مجموعة التحكم ؛ مجموعة المعالجة بـ بمستخلص القسط (200 ملغ /كغ من وزن الجسم)؛ مجموعة المعالجة بعقار التامكسوفين (40 ملغ/كغ من وزن الجسم)؛ مجموعة المعالجة بالتامكسوفين بالتزامن مع المعالجة بالمستخلص النباتي المائي . كانت كل المعالجات مأخوذة بفعل الإنبوب المعدي لمدة 28 يوم متتاليا . أظهرت النتائج ان الجرذان المعالجة بعقار التامكسوفين TAM زادت بشكل ملحوظ من مؤشرات وظائف الكلى في المصل، وهي الكرياتينين، واليوريا، وحمض اليوريك، مع انخفاض متزامن في الاليكتروليتات في المصل وهي (Na+، K+، CL -) مقارنة بمجموعة التحكم . أدى تناول المستخلص المائي لجذور القسط الهندي بالتزامن مع التامكسوفين إلى تحسين مؤشرات وظائف الكلى بالإضافة إلى مستويات الاليكتروليتات في المصل مقارنة بالحيوانات التي تعرضت لتسمم TAM .تم تأكيد هذه النتائج من خلال الفحص النسيجي لقطاعات الكلي، والذي اظهر ضمور الكبيبات الكلوية وانحلال خلايا الظهارة الأنبوبية.

قد يكون للمستخلص الجذري المائي لنبات القسط الهندي (AESL) قدرة وقائية لحماية الكلى من الضرر الناتج عن تأثير سمية عقار التامكسوفين TAM .

الكلمات المفتاحية: التسمم الكلوي ، التامكسوفين ، مستخلص القسط الهندي ، اناث الجرذان

Abstract

Worldwide, breast cancer is the most common tumour that affecting mostly women. Tamoxifen (TAM) is frequently employed as the initial treatment for people diagnosed with breast cancer. However, research has demonstrated that prolonged use of this medication might lead to nephrotoxicity. The purpose of this study is to assess the nephroprotective impact of the aqueous root extract of *Saussurea lappa* (AESL) in mitigating renal damage generated by TAM.

Rats were divided mainly into four groups: normal control group; AESL(200 mg/kg body weight) treated group; TAM (40 mg/kg) administered group; and TAM concurrently with AESL treated group. All treatments were given through a gastric tube for 28 days. The data revealed that oral administration of TAM, significantly increased serum renal function markers, namely, creatinine, urea, and uric acid with a concomitant depletion in serum electrolytes (Na+, K+ and Cl^-) in TAM intoxicated rats with respect compered to control group. Oral ingestion of AESL with TAM, successfully improved renal function markers and serum electrolyte levels in rats compared to TAM-intoxicated rats. Histopathological analysis confirmed these findings, which showed glomerular atrophy and tubular epithelial cell degeneration. Supplementation with AESL may have the ability to protect the kidney from damage caused by TAM toxicity.

Keywords: Nephrotexocity, tamoxifen, *Saussurea lappa* extract, female rats

Introduction

Tamoxifen (TAM) is an antiestrogen, widely used in the treatment of breast cancer **Shukla et al., 2016:7-11)**. TAM, although effective in reducing breast cancer in high-risk women by 49%, its toxicity towards multiple organs and tissues including kidney is a well-established **(Tabassum et al., 2007: 509-518)**. It has reported that administration of antineoplastic drugs or adjuvant therapy, such as TAM, has been found to directly result in a decrease in renal functions **(Kintzel and Dorr., 1995:33-64).** Generally, the kidney is commonly regarded as the essential organ responsible for the removal of drug poisons, and naturally produced metabolites **(Torres, 2008:6616).** It was stated that the carcinogenic effect of TAM is due to its metabolites forming covalent bonds with DNA, leading to the development of DNA adducts in the renal cells **(Li et al., 1997: 1438-1441).** TAM has a significant role in causing uterine and liver cancer, as well as

thromboembolic diseases in both humans and rats **(Kärki et al., 2000:246-256)**. On the other hand, studies have suggested that oxidative stress and production of reactive oxygen species (ROS) are considered as a causative agents of TAM – induced renal tissue damage **(Zuhair 2011:15)**. ROS produced by TAM, such as superoxide anions (O2-), hydroxyl radical (OH·), and hydrogen peroxide (H2O2), can cause tissue damage via supressing the antioxidant capacity of the tissue **(Zuhair 2011:15)**, **(Saad and Al-Rikabi., 2002:42-48)** Several naturally occurring phytochemical compounds are still regarded as the most promising choices in protecting against tissue damage due to their wide range of biological and therapeutic uses **(Abdallah et al., 2017:601-611)**.

Saussurea lappa is a widely used plant in several therapeutic applications. The main chemical compounds found in *S. lappa* are sesquiterpene lactones, including, dehydrocostus lactone, costunolide, and cynaropicrin **(Abd El-Rahman et al., 2020:396)**. The primary constituents of the essential oil derived from the roots of *S. lappa* are sesquiterpenoids, which make up around 79.80% of its overall makeup **(Hanh et al., 2021:1399-1405)**. The roots of *S. lappa* have been demonstrated to be rich in acetylated flavone glycosides, palmitic and linoleic acids, and chlorogenic acid **(El Gizawy et al., 2022:2802)**. Recent studies have shown that these metabolites have a range of therapeutic activities, including antifungal **(Barrero et al., 2000:60-64)**, anti-diabetic, anti-cancer, antiprotozoal **(Ko et al., 2005:11-19)**, immunostimulant **(Kulkarni and Desai., 2001:292-294),** antiulcer **(Sutar et al., 2011:516-520)** antimicrobial **(Khalid et al., 2011:4574-4580),** anti-inflammatory **(Sunkara,Y et al .,2010:1775–1778)**, hepatoprotective and renoprotective activities (**Khalid et al., 2011:4574-4580)** ,**(Elshaer et al., 2024:15)** .

The purpose of this study was to determine the nephroprotective effect of *Saussurea lappa*'s aqueous root extract (AESL) against TAM-induced nephrotoxicity.

Materials and methods

Chemicals

Tamoxifen (tamoxifen citrate), trade name Nolvadex®, was manufactured and packed as tablets by Astra Zeneca, United Kingdom. It was suspended in distilled water and orally given to the experimental animals at dose level of 40 mg/kg body weight which equivalent to the therapeutic dose for human, according **to Paget and Barnes. 1964:135-65** daily for 28 days.

Plant material and extraction method for *Saussurea lappa* (AESL)

S. lappa dry roots were obtained from medicinal plant market, Benghazi, Libya. To prepare the aqueous root extract, one kg of *S. lappa* root was powdered, boiled for 30 minutes with 5 litres of distilled water and then filtered. The obtained extract was then lyophilized. For this investigation, the freeze-dried substance was weighed (about 35 g), and dissolved in water to get a final concentration of 50 mg/ml **(Saleem et al., 2013:94-100)**.

Animals and Treatment

Twenty four females of albino rats (eight weeks age), weighing 180-190 g, were utilized for this work. The rats were obtained from animal house, University of Benghazi, Faculty of medicine. The animals were housed under controlled conditions (23-25 ºC, humidity 50-65%, 12 h dark/light cycles). Animals were given a diet with standard composition and water adlibitum. The animals were left for seven days for adaptation and then distributed into four groups, 6 rats each group.

Group I: Normal animals treated orally with normal saline only.

Group II: rats treated orally with SLRE (200 mg/Kg /day) for 28 consecutive days **(Saleem et al., 2013:94-100).**

Group III: Rats treated orally with a suspension of TMX (40 mg/Kg/day) for 28 consecutive days **(Gudbrandsen et al., 2006: 2223-2232).**

Group IV: rats received orally TMX (40 mg/Kg/day) concurrently with (AESL) (200 mg/Kg /day) for 28 consecutive days.

After the experimental period, the blood was collected in a sterile and dry centrifugal tube and allowed to rest for 30 minutes before being centrifuged to prevent hemolysis. The samples underwent centrifugation for duration of 15 minutes at a speed of 2,500 revolutions per minute. The transparent serum was separated and gathered using a Pasteur pipette into a sterile tube for subsequent biochemical analyses. The animals were then scarified and the kidneys were taken, washed with normal saline and weighed for biochemical analysis and histopathological examination.

Biochemical analysis

Serum analysis

Creatinine, urea, uric acid and electrolyte levels (potassium, sodium, and chloride) were determined by using commercial kits (Sigma-Aldrich).

Histopathological investigation

A small portion of kidney tissue obtained from each rat was fixed in 10% aqueous formalin solution, washed with 70% ethanol, dehydrated using alcohol series from 70% to 100% alcohol and embedded in paraffin. The paraffin sections were stained with haematoxylin and eosin (H&E) dyes and observed under a light microscope **(Stevens and Bancroft., 1990)**

Statistical analysis

Data were analyzed by comparing the mean values for different TMX groups with the mean values of controls. Results are expressed as mean ± SD. Significant differences among values were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's test post-ANOVA. Values were considered statistically significant at $p \leq 0.05$. All statistical analysis was done by SPSS 12 softwear.

Results

Serum kidney function markers

The efficacy of *S. lappa* root extract (AESL) on serum renal function markers (creatinine, urea and uric acid) in TMX induced renal damage in rats was illustrated in **Table 1**. Non significant difference in these renal damage markers was observed in rats treated with AESL only versus control animals. However, elevation in serum creatinine, urea and uric acid levels was recorded in TMX treated rats with respect to control ones ($p \leq 0.001$). Administration of SLRE concurrently with TMX, markedly down modulated the serum levels of renal function indices versus TMX untreated group ($p \leq 0.001$).

Table 1: Impact of aqueous root extract of *Saussurea lappa* on serum kidney function markers in TMX- induced nephrotoxicity in rats.

Parameters	Control	*S. lappa*	TMX	TMX+AESL
Creatinine (mg/dl)	1.40±0.04	1.37±0.03*	3.18±0.025[a]	1.95±0.17 [a*]
Urea (mg/dl)	31.5±1.87	31.33±2.80*	121.16±2.78[a]	66.83±3.97 [a*]
Uric acid (mg/dl)	1.34±0.020	1.31±0.023*	4.15±0.43 [a]	2.20±0.030 [a*]

Values are expressed as mean ± SD of 6 rats. [a]$p \leq 0.001$ compared with control, *$p \leq 0.001$ compared with TMX. **(عمل الباحثة)**

Serum electrolytes disorder

Table2 shows the effect of AESL on serum electrolytes (Na^+, K^+ and Cl^-) in TMX induced renal damage in rats. Non significant changes in these electrolytes were noticed in rats treated with AESL only versus control animals. However, depletion in serum Na^+, K^+ and Cl^- levels was recorded in TMX treated rats with respect to control ones ($p \leq$

0.001) . Administration of AESL concurrently with TMX, markedly up modulated the serum levels of these electrolytes versus TMX untreated group ($p \leq 0.001$).

Table 2: Impact of aqueous root extract of *S. lappa* on serum electrolytes in TMX-induced nephrotoxicity in rats.

Parameters	Control	*S. lappa*	TMX	TMX+AESL
Na+ (mmol/L)	130.50±1.87	130.16±2.78*	67.00±4.28[a]	88.5±3.50 [a*]
K+ (mmol/L)	11.13±0.29	11.10±0.41*	5.08±0.19[a]	7.93±0.43 [a*]
Cl^- (mmol/L)	102.33 ±4.50	100.16±2.93*	30.00±2.61[a]	83.33±4.50 [a*]

Values are expressed as mean ± SD of 6 rats. [a]$p \leq 0.001$ compared with control, *$p \leq 0.001$ Compared with TMX(عمل الباحثة) .

Histopathological observation

Kidney sections of control rats as well as rats treated with AESL only exhibited normal renal architecture with normal glomeruli (**Figure 1a** & **1b** respectively). Rats received TAM (**Figure 1c)** showed histo-morphological changes and marked injury as evident by atrophy of most glomeruli with epithelial degeneration of most tubules. Administration of TAM concurrently with AESL (**Figure 1d**) showed normal kidney architecture with glomeruli.

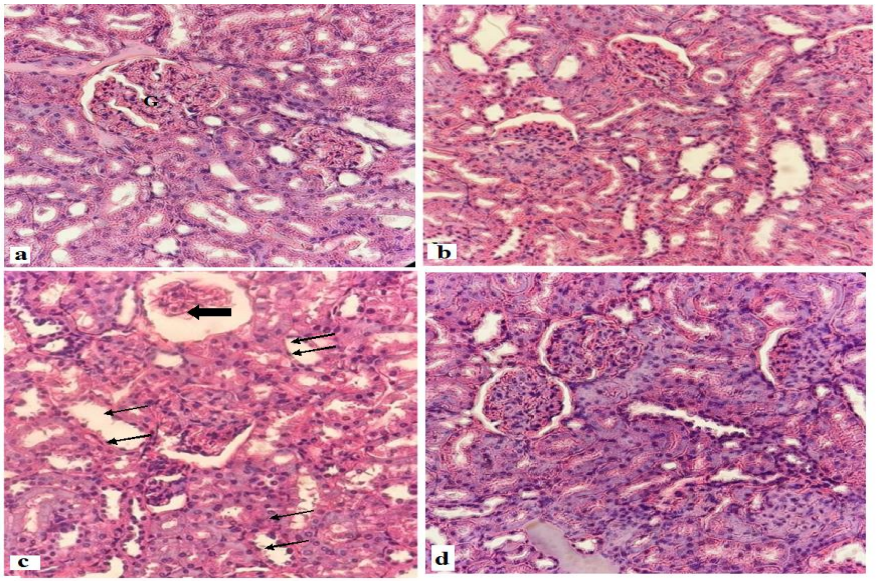

Fig 1 : Light micrograph of rat kidney sections of TAM treated groups stained with H&E. (a) kidney section of control rat, showing normal kidney architecture with normal glomeruli (G); (b) kidney section of rat treated with AESL only showing normal kidney

architecture and normal glomeruli ; (c) kidney section of TAM treated rat, showing degenerative glomerular tuft with capillary loss (thick arrow) , atrophy of tubular cells (thin arrows); (d) kidney section of TAM treated rat concurrently with AESL showing normal kidney architecture (H&E, X400). **(عمل الباحثة).**

Discussion

The occurrence of kidney failure is primarily linked to chemotherapy-induced renal impairment

(Liangos et al., 2006:43-51). Chronic renal damage can result in the progression to end-stage renal disease **(Liangos et al., 2006:43-51)**.

TAM is widely used as chemotherapy for breast cancer. However, its side impact on the other organs is of main importance in its clinical usage (**Tabassum et al., 2007: 509-518)(**Torres**, 2008:6616)**. It has been reported that treatment with this drug causes oxidative stress, inflammation and apoptosis, leading to tissue damage **(Lippman and Brown., 1996: 1809-1819)** . **(Lien et al., 1991: 4837-4844)** stated that the kidney is one of the organs with high affinity to TAM and its metabolites. There is a scarcity of researchers who discuss the impact of TAM on kidney function tests. Thus, the aim of this research was to explore the prophylactic influence of AESL on nephrotoxicity related to TAM therapy in female rats.

In the present study, administration of TAM (40 mg/kg) for 28 successive days provoked nephrotoxicity as shown by the increment in the levels of serum creatinine, urea and uric acid. This result may give a clue that this drug caused disruption of renal blood flow and glomerular filtration rate. The adverse influence of TAM was further ensured by histological observation as observed by glomerular atrophy, degeneration of tubular epithelial cells. These findings are consistent with **(Ahmed et al., 2008:370-376)**, who reported that oral TAM therapy is linked to impaired kidney function tests, possibly due to damage to the vasculature or structures of the kidneys. It has been reported that oxidative stress, a result of excessive production of reactive oxygen species (ROS) and a disruption in the biological defence system, is a significant contributor to various human complications **(Tabassum et al., 2007: 509-518)**. There is a growing body of research indicating that TAM stimulates the generation of reactive oxygen species (ROS) and depletes the cellular thiol system, which are significant factors in causing kidney damage

(Parlakpinar et al., 2005:169-177). ROS interact with the mitochondrial membranes in the kidneys, resulting in the loss of the structural integrity of the kidneys **(Tabassum et al., 2007: 509-518)**. In addition, **El-Beshbishy in 2005: 563-570** reported that the administration of TAM resulted in an increase in lipid peroxidation, leading to a reduction in the levels of several intracellular antioxidant enzymes as well as nicotinamide adenine dinucleotide phosphate (NADPH), which plays a role in defending cells against ROS **(Diplock, Rice-Evans, and Burdon 1994:1952s-1956s).** Besides, **Nazarewicz et al., 2007: 1282-1290)** stated that TAM can induce the production of mitochondrial nitric oxide (NO), which further oxidized to very potent oxidant known as peroxynitrite. Generation of such oxidant can cause damage to the blood vessels and the kidney structures. The present alteration in the histo-morphologic picture of kidney tissue in rats under the effect of TAM administration may indicate the severe pathological impact of TAM, which in turn dramatically affect the kidney function by increasing the levels of endogenous end products such as creatinine, urea, and uric acid in the bloodstream **(Ahmed et al., 2008:370-376).**

Co intake of AESL with TAM markedly hampered the alteration in renal function parameters as well as histopathological changes in renal architecture. This result is an index of the protective impact of AESL on the nephrotoxicity induced by TAM therapy. The observed nephroprotective impact of AESL aligns with previous research that has demonstrated the nephroprotective capabilities of *S. lappa* extract in mitigating kidney damage generated by paracetamol. This beneficial effect of the plant extract may be attributed to its phytochemical compounds such as dehydrocostus lactone, costunolide, cynaropicrin, monoterpenes, sesquiterpenoids, flavonoids, lignans, triterpenes, steroids, and glycosides, which exhibit anti-inflammatory, antioxidant and/or free-radical scavenging activities **(Ayaz, N. 2017:2) (Kadhem, M. 2019: 68-73)**. This suggests that they have the ability to reduce kidney damage **(Wang et al., 2014:2085-2090)**. Also, it has found that γ -linolenic acid, a prominent metabolite detected in *Saussurea* ethanolic extract, exhibited antioxidant properties and possible nephroprotective effects **(Teng et al., 2017:418-424)**. Furthermore , the presence of D-(+)-malic acid in the plant extract with its known hepatoprotective properties, may highlights the potential nephron-protective value of this extract **(Koriem and Tharwat, 2023:98)**.

Electrolyte abnormalities is the main character of renal disorder **(Piccoli et al., 2010:247)** The current investigation showed that administration of TAM caused hypokalaemia, hyponatremia, and hypochloraemia in TAM intoxicated rats compared with control ones. To the best of our information, there is no previous study on the impact of TAM on serum electrolytes. So the present result may suggest that the abnormalities in the serum electrolytes presented in the current study in TAM intoxicated rats may attribute to impaired renal dilution capability and/ or the damaging effect of TAM on renal tubules and glomerular filtration rate as well as capillary loss. This is confirmed by the present renal dysfunction with histopathological alteration in rats under the effect of TAM toxicity. On the basis of both animal and human studies, **(Reungjui et al., 2008125-134)** Presumed that the pathogenesis of hypokalaemic nephropathy is due to progressive capillary loss, reduced endothelial cell proliferation, and loss of vascular endothelial growth factor expression. In addition, some authors revealed that the increased renal ammonia genesis **(Tizianello et al.,1991:772-778)** activating the alternative complement pathway and increased profibrotic cytokine expression (insulin-like growth factor-1 and transforming growth factor) **(Suga et al., 2003: 397-406)**. Also, **(Agrawal et al., 2008: 956-64)** reported that hypernatremia could indicate an impaired renal dilution capability due to renal damage. The same authors added that hypernatremia can be caused due to impaired tubular reabsorption and osmotic disequilibrium between the luminal fluid and medullary interstitial impairs dilution.

Intake of AESL with TAM effectively ameliorated the alteration in serum electrolytes compared to TAM intoxicated rats. This result may confirm the protective impact of the active constituents of the used plant extract against renal damage in rats under the effect of TAM toxicity.

Conclusion

The modulating effect of *S. lappa* extract on renal function biomarkers and its histomorphological picture may suggest the reno-protective effect of this plant extract against TAM induced renal damage. This beneficial effect of the plant extract may ascribe to the ability

of its active compounds to mitigate renal damage due to TAM toxicity .

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