Therapeutic Effect of Costus (*Saussurea lappa*) Root Aqueous Extract on Tamoxifen-Induced Fatty Liver in Female Rats

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Abstract

Hepatic damage is a well-known adverse consequence of tamoxifen treatment for breast cancer patients. *Sauusurea lappa* (costus) is a therapeutic herb used in traditional medicine to treat hepatic ailments. The study aimed to evaluate the potential hepatoprotective effects of *Saussurea lappa* root extract (SLRE) against tamoxifen-induced liver damage in rats. Four groups of female *Wistar albino* rats were assigned to different treatments (six females each) for duration of twenty eight days. Group1, rats administered orally with normal saline alone and treated as a control group. In group 2, rats received oral administration of SLRE (200 mg/ kg body weight/day).. Group 3, rats administered TMX orally at dosage of 40 mg/ kg body weight every day. Group 4, rats were administered orally with TMX concurrently with SLRE The data showed that treatment with SLRE significantly reduced the serum levels of triglyceride (TG), total

cholesterol and low-density lipoprotein-cholesterol (LDL-C) with non significant changes in ALT , AST, ALP , albumin, total protein and glucose were recorded in comparison to control animals. Co-administration of SLRE to TMX intoxicated rats, significantly down-modulated the elevation of serum TG, ALT, AST, and glucose levels versus TMX treated group. However, non significant change was shown in ALP level in TMX - SLRE treated group versus TMX group. Also, the result revealed that the intake of the plant extract to TMX group, markedly up modulated the serum albumin, total protein, and HDL-C levels and down-regulated the serum total cholesterol and glucose content versus TMX intoxicated group. The present biochemical investigation was documented by histopathological study.

Keywords*:* *Saussurea lappa*, tamoxifen, rats, liver damage.

تعتبر الأضرار الكبدية نتيجة سلبية معروفة لعلاج التاموكسيفين لمرضى سرطان الثدي . القسط الهندي ***Saussurea lappa*** هو نبات طبي، يستخدم في الطب التقليدي لعلاج اضطرابات الكبد الناتجة عن العلاج بالتامكسوفين **(TAM)** .هدف دراستنا كان تقييم التأثيرات المحتملة لحماية الكبد من الأثار الجانبية لعقار التامكسوفين بفعل المستخلص المائي لجذورنبات القسط الهندي (**SLRE**). تم تقسيم أربع مجموعات من إناث الجرذان (6 جرذان لكل مجموعة) بوزن 180-190 جرام إلى علاجات مختلفة، المجموعة الأولي (الكنترول أو الضابطة) التي تم علاجها عن طريق الفم بمحلول ملحي عادي فقط ، (المجموعة الثانية) مستخلص القسط الهندي(SLRE) ، تم إعطاء الجرذان عن طريق الفم بفعل الإنبوب المعدي (200 ملغ/كغ من وزن الجسم/يوم) من المستخلص النباتي لمدة 28 يومًا متتاليًا، (المجموعة الثالثة)(مجموعة التامكسوفين TAM) تم إعطاء الجرذان عن

طريق الفم معلق التامكسوفين (40 ملغ/كغ من وزن الجسم/يوم) لمدة 28 يومًا متتاليًا؛ المجموعة الرابعة (التامكسوفين + المستخلص النباتي)، تم إعطاء الجرذان عن طريق الفمTMX بالتزامن مع SLRE لمدة 28 يومًا متتاليا. أظهرت البيانات أن معالجة االجرذان في المجموعة الثانية (مستخلص القسط الهندي SLRE) أدى إلى تقليل ملحوظ في مستويات السيروم من الدهون الثلاثية (TG) والكوليسترول الكلي والكوليسترول الدهني منخفض الكثافة(LDL-C) ، بينما لم تُسجل تغييرات ذات دلالة إحصائية في الانزيمات الكبديةALT ،AST، ALP والألبومين والبروتين الكلي والجلوكوز مقارنة بالحيوانات الضابطة، لوحظ في المجموعة الرابعة (SLRE+ TAM) انخفاض بشكل ملحوظ في مستويات كلا من TG ALT، AST، والجلوكوز في المصل مقارنةً بمجموعة الفئران المعرضة لتسمم TMX ومع ذلك، لم يظهر تغيير ذو دلالة في مستوى ALP في مجموعة المعالجة بـ TMX + SLRE مقارنةً بمجموعة TMX ,كما كشفت النتائج أن تناول مستخلص النبات لمجموعة TMX زاد بشكل ملحوظ من مستويات الألبومين في المصل، والبروتين الكلي، ومستوياتHDL-C ، بينما خفض من مستوى الكوليسترول الكلي في المصل والجلوكوز مقارنةً بمجموعة TMX المسمومة. تم توثيق نتائج البيوكيميائي الحالي من خلال دراسة الأنسجة المرضية.

تقترح النتائج الحالية أن العلاج الوقائي باستخدام المستخلص المائي لجذورنبات القسط الهندي SLRE يمكن أن يُعتبر علاجًا وقائيًا ضد التسمم الكبدي الناتج عن الأدوية مثل عقار التامكسوفين .

1 Introduction

Hepatic steatosis is one of the chronic liver illnesses that affects 10–24% of people worldwide. It characterized by the pathologic accumulation of intrahepatic fat **(1)**. Although the condition first manifests as benign steatosis, it can eventually lead to steatohepatitis, fibrosis, cirrhosis, and hepatocellular cancer. Steatotic liver damage can be brought by the following mechanisms: (a) enhanced the absorption of free fatty acids (FFA) via hepatocytes from dietary fats; (b) increased de novo lipogenesis (DNL); (c) reduced the mitochondrial oxidation of fatty acids; and (d) decreased lipid production derived from hepatocytes as very low density lipoprotein (VLDL) **(2).**There are several risk factors that can cause steatotic liver damage, including obesity, metabolic disorders, and some pharmaceuticals **(3)**. Tamoxifen, often known as Nolvadex (TMX, 2-[4- [(Z)-1,2-diphenylbut-1-enylphenoxy]-N,N-dimethylethanamine) **(4)**, is a medicine that could precipitate fatty liver **(5,6)**.

Furthermore, TMX is an artificial non-steroid prescription medicine that is frequently used to treat patients with estrogen receptor-positive breast cancer. Although its beneficial impacts in the treatment of breast cancer, this drug showed hepatic damage in the form of enhancement of hepatic function measures, jaundice, steatohepatitis, and hepatic necrosis **(7),** as well as cirrhosis **(8)**, Numerous studies have examined the methods via which TMX causes liver injury. Researchers have demonstrated that TMX induces hepatic

fat accumulation by enhancing the production of triglycerides. **(9)**. Inhibiting oxidation of fatty acids also reduces liver triglyceride level production which TMX causes liver injury **(10)**. Researchers have demonstrated that TMX induces hepatic fat accumulation by enhancing the production of triglycerides. **(9)**. Inhibiting oxidation of fatty acids also reduces liver triglyceride level production **(10)**. The modification in lipid metabolism associated with TMX-induced steatotic liver injury is significantly correlated with the dosage (0.5 - 200 mg/kg/day) and the period of display (5 - 28 days) **(10)**. Inflammation and oxidative stress are the primary mechanisms via which TMX promotes NASH **(11,12)** have reported these findings.

A wide range of clinical conditions are being treated using herbal medications made from plant extracts, which are becoming more and more popular because of their low cost, accessibility, and lack of adverse effects *Saussurea lappa* is a significant therapeutic plant. This plant showed many therapeutic activities. These include anti-diabetic, anti-hepatotoxic, anti-inflammatory , anthelmintic, antifungal, antitumor, anti-ulcer, antioxidants ,antibacterial, and immunostimulatory characteristics **(13,14).** *S. lappa* has hypolipidemic properties, diminishing plasma whole cholesterol, and triglyceride levels while augmenting activities of liver antioxidant enzymes **(15)**. The main active chemicals from *S. lappa* with potential therapeutic qualities, include costunolide and dehydrocostus lactone **(16)**.

The current study investigated, for the first time, the avoidance impacts of SLRE in comparison to TMX hepatotoxicity. The goal of the investigation was to explore the potential effects of *S. Lappa* root water extract (SLRE) on steatotic liver injury induced by tamoxifen.

2 Materials and Methods

Chemicals

Tamoxifen: AstraZeneca produced Tamoxifen (tamoxifen citrate) in the United Kingdom and packaged it as tablets under the brand name Nolvadex®. We solubilized the compound in distilled water and administered it orally to the experiment animals at a dosage of 40 mg/kg of body weight, which aligns with the recommended human dosage. We administered the compound daily for a period of 28 days **(17).**

Plant material and extraction method for *Saussurea lappa*

I obtained *S. lappa* dry roots from medicinal plant markets in Benghazi, Libya. To prepare the aqueous root extract, one kg of *S. lappa* root was finely powdered, boiled for 30 minutes with 5 litres of distilled water, and subsequently filtered. The extracted substance was subsequently lyophilized. In this experiment, we weighed about 35 g of the dried material and dissolved it in water to achieve an end concentration of 50 mg/mL **(18).**

Experimental design

Twenty-four female albino rats, aged eight weeks and weighing between 180 and 190 g, were employed for this research. I obtained all the rats from the animal house at the Faculty of Medicine,

University of Benghazi. The rats were kept in controlled environments with temperatures ranging from (23-25℃, humidity from 50 - 65 %, and a 12-hour cycle of darkness and light. I provided them with a standard composition diet and unrestricted water consumption. I gave the rats a week to acclimate to their environment before dividing them into four distinct categories, each containing six rats.

Group I: Standard animals administered alone orally with normal saline.

Group II: For 28 consecutive days, rats received SLRE orally at a dose of 200 mg/kg/day (18).

Group III: The rats administered TMX suspension (40 mg/kg/day) orally to the rats for 28 consecutive days (19).

Group IV: For 28 consecutive days, rats received TMX (40 mg/kg/day) and SLRE (200 mg/kg/day) orally.

Following the experiment phase, rats underwent an overnight fasting that lasted 12–14 hours. I collected and used a centrifuge to the blood samples for coagulation and serum separation. After euthanizing the animals, I removed their livers, washed them in normal saline, and evaluated them for biochemical assays and histological examination.

Biochemical analysis

Serum analysis

Total cholesterol, serum triglyceride, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST),

and alkaline phosphatase (ALP), total protein, albumin, and glucose were investigated using commercial kits (Sigma-Aldrich).

Histopathological examinations of hepatic tissues

A small sample of liver tissue from each rat was preserved in 10% diluted formalin solution, rinsed with 70% ethanol, dehydrated through a range of alcohol concentrations of 70 % to 100%, and subsequently embedded in paraffin. I stained the paraffin blocks with haematoxylin and eosin (H&E) and examined them under a light microscope (Harris,1959).

Statistical analysis

I examined the data by comparing the mean outcomes of numerous TMX groups to the mean values of a control group. Presentations of results show means ± the standard deviation (SD). A one-way analysis of the variance (ANOVA) and then Bonferroni's post hoc test evaluated notable variances in values. At $p < 0.05$, values were judged to have statistical relevance. The statistical analysis were done using SPSS 12 software.

3 Results

Serum liver function markers

The influence of *S. lappa* root extract (SLRE) on serum liver function markers (ALT, AST, and ALP) in TMX-induced liver damage in rats was illustrated in **Table 1**. Rats treated with *S. lappa* extract only showed nonsignificant variation in ALT, AST, and ALP compared to control animals. Elevation in serum ALT, AST, and ALP levels in TMX-treated rats with respect to control ones ($p \leq 0.001$).

The administration of the plant extract (SLRE) to the TMX group significantly reduced the levels of ALT and AST.

TMX untreated group ($p \leq 0.001$), however, no significant change was shown in ALP level in the TMX *S. lappa* extract-treated group versus the TMX group .

Table 1: Impact of Aqueous Extract root *Saussurea lappa* on serum liver function markers in TMX -induced liver damage in rats

Parameters	Control	*S.Lappa*	TMX	TMX+(SLRE)
ALT (U/L)	56.17± 2.639	57.50± 3.728*	100.80± 5.269 [a]	76.83± 3.312 [a*]
AST (U/L)	90.67± 3.38	90.17± 2.78*	140.5± 3.83 [a]	120.8± 2.48 [a*]
ALP (U/L)	310± 39	320± 4.5*	390± 2.6 [a]	370± 4.9 [a]

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

Serum proteins

Table 2 records the influence of SLRE on serum albumin and total protein levels. Rats treated with SLRE alone showed no significant difference in these proteins compared to control animals. Albumin and total protein levels were much lower in rats that had been given TMX than in rats that had not been given TMX ($p \leq 0.001$). Administration of the plant extract to the TMX group markedly up modulated the serum albumin and total protein levels versus the TMX-intoxicated group ($p \leq 0.001$).

Table 2: Impact of Aqueous Extract Coustus *Saussurea lappa* on serum proteins levels in TMX -induced liver steatosis in rats

Parameters	Control	*S .Lappa*	TMX	TMX+(SLRE)
Albumin (g/dl)	6.10± 0.28	6.30± 0.41*	3.30± 0.22 [a]	5.00± 0.45 [a*]
T. Protein (g/dl)	7.96± 0.21	8.08± 0.45*	4.01± 0.24 [a]	5.25± 0.48 [a*]

Values are expressed as mean ± SD of 6 rats. [a] p≤ 0.001 compared with control group, *p≤0.001 compared with TMX group.

Serum lipid profiles

Figures 1, 2, 3, and **4** show the impacts of SLRE on serum lipid indices, including serum triglycerides (TG), total cholesterol, LDL-C, and HDL-C in rats with TMX-induced liver damage. The results indicated that the treatment of normal rats with *S. lappa* root extract significantly reduced the serum levels of TG, T cholesterol, and LDL-Ccomparison to control animals (p≤ 0.05 for TG and LDL-C, p≤ 0.001 for T cholesterol).

Oral treatment of female rats with TMX for 28 successive days significantly boosted the level of TG versus control rats (p≤ 0.001), and oral co-administration of SLRE root extract significantly reduced the TG level versus the TMX-treated group (p≤ 0.001). Meanwhile, significant depletion in T. cholesterol, LDL-C, and HDL-C was observed in TMX-treated rats with respect to control animals (p≤ 0.001 for T. cholesterol and HDL-C, p≤ 0.05 for LDL-C). Treatment with the plant extract simultaneously with TMX markedly ameliorated

serum T. cholesterol and HDL-C versus TMX-intoxicated rats (p≤ 0.001, p≤ 0.01, respectively), while no significant changes in LDL-C levels were recorded in TMX-SLRE-treated rats versus TMX-untreated ones.

Serum glycemic index

Figure5 demonstrates the level of serum glucose (glycemic index) in different animal groups. Non-significant variation in serum glucose level in animals ingested SLRE only versus control counterparts, while elevation in this glycemic index in TMX-intoxicated rats in comparison to control ones (p≤ 0.001). Administration of SLRE concurrently with TMX markedly ameliorated serum glucose concentration in the TMX-SLRE-treated group with respect to the TMX-intoxicated group (p≤ 0.001).

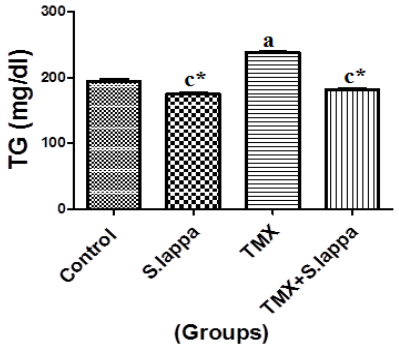

Fig. 1: Effect of SLRE on serum TG in TMX-induced liver damage in rats. Values are expressed as mean ± SD of 6 rats. ^{a}p≤ 0.001, ^{c}p≤ 0.05 compared with control, *p≤0.001 compared with TMX

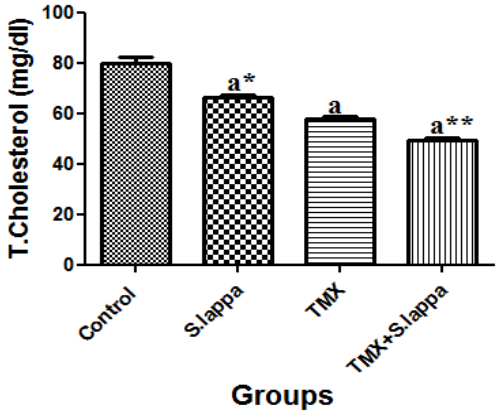

Fig. 2: Effect of SLRE on serum T cholesterol in TMX-induced liver damage in rats. Values are expressed as mean ± SD of 6 rats. $^{a}p\leq 0.001$, $^{c}p\leq 0.05$ compared with control, *$p\leq 0.001$, **$p\leq 0.01$ compared with TMX.

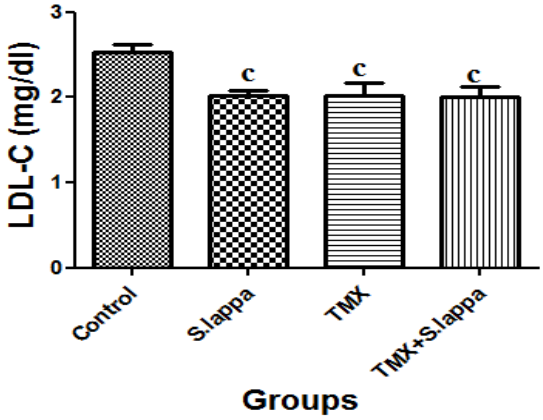

Fig. 3: Effect of SLRE on serum LDL-C in TMX- induced liver damage in rats. Values are expressed as mean ± SD of 6 rats. $^{c}p\leq 0.05$ compared with control.

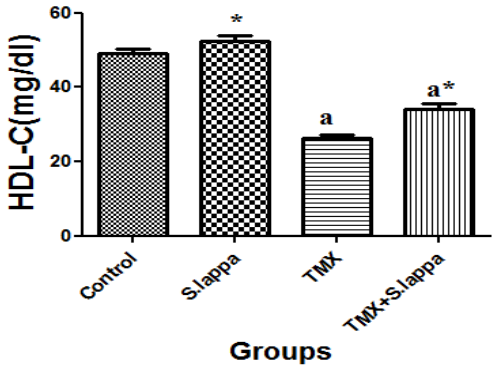

Fig.4: Effect of SLRE on serum LDL-C in TMX- induced liver damage in rats. Values are expressed as mean ± SD of 6 rats. $^{a}p\leq 0.001$ compared with control, *$p\leq 0.001$ compared with TMX.

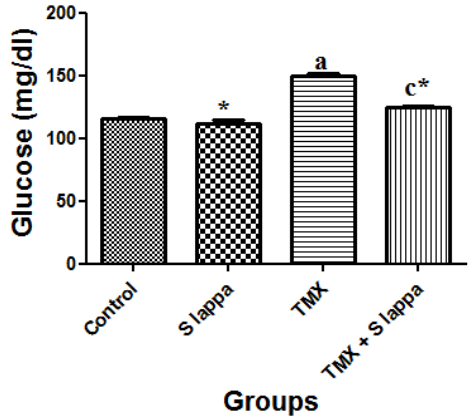

Fig.5: Effect of SLRE on serum glucose in TMX -induced liver damage in rats. Values are expressed as mean ± SD of 6 rats. $^{a}p\leq 0.001$, $^{c}p\leq 0.05$ compared with control, *$p\leq 0.001$ compared with TMX.

Histopathological result

Histological observation of liver sections of control rats and rats treated with SLRE only (**Figures 6 and 7**, respectively) revealed normal liver architecture with normal hepatocytes. Liver sections of

rats treated with TMX showed many histopathological changes, including disorientation of tissue architecture and vesicular steatosis (**Figure 8**). Many hepatocytes appear with pyknotic nuclei (**Figure 8**), and others with devoid nuclei (**Figure 9**). Infiltration of inflammatory cells was also observed (**Figure 9**). Liver sections of rats treated with SLRE in parallel with TMX showed great normal liver architecture with normal hepatocytes with no vesicular steatosis (**Figure 10**).

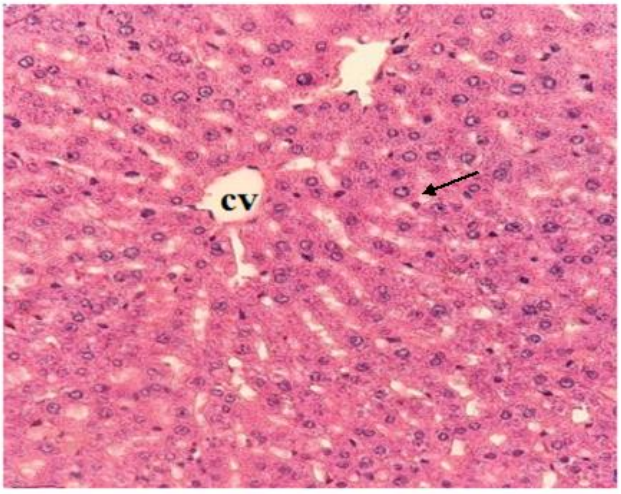

Figure 6. Light micrograph of normal rat liver section, showing classical hepatic lobules. The hepatocytes appeared polyhedral in shape (arrow) with acidophilic cytoplasm around the central vein (CV) and rounded vesicular nuclei. (H&EX400).

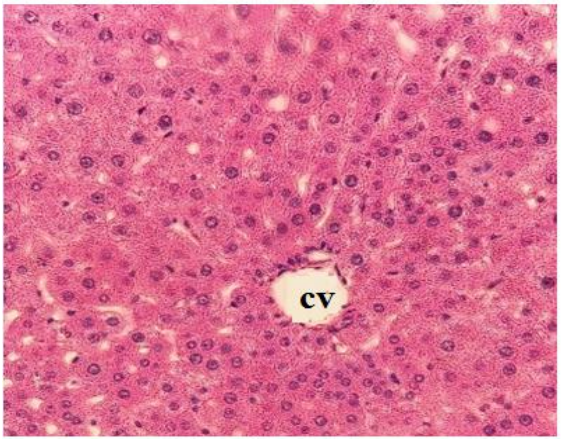

Figure 7 : Light micrograph of liver section of rat treated with SLRE only , showing normal liver architecture with normal hepatocytes (H&EX400).

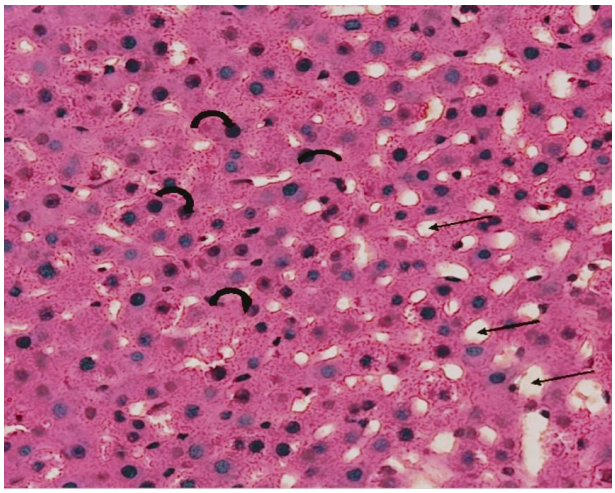

Figure 8 : Light micrograph of liver section of rat treated with TMX Showing, vesicular steatosis (black thin arrows), many hepatocytes with pyknotic nuclei (circular arrows) and other cells appear with devoid nuclei (H&EX400).

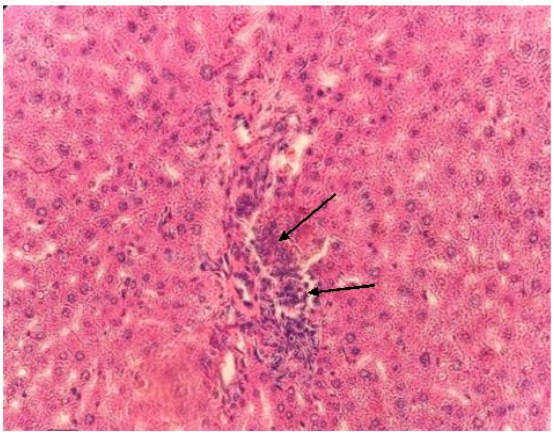

Figure 9 : Light micrograph of another liver section of rat treated with TMX Showing disorientation of hepatocytes, infiltration of inflammatory immune cells and hepatocytes with devoid nuclei (curved arrows).

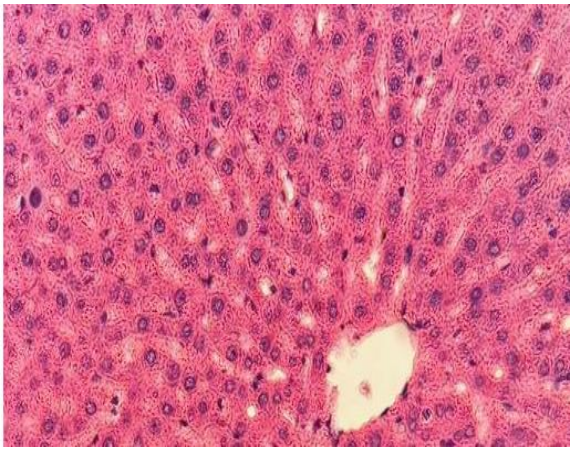

Figure 10 : Light micrograph of liver section of rat treated with SLRE concurrently with TMX , showing normal liver architecture with normal hepatocytes (H&EX400).

4 Discussion

Pharmaceuticals are becoming recognized as a major contributor to liver damage in the general populace, and drug induced liver injury (DILI) is presently a leading cause for the removal of medications in clinical practice.

TAM is a harmful medicine for the tissue of the liver because of its stronger perfection for the patocytes than for other cells, which causes liver impairment as one of the adverse effects of women with breast cancer who are treated with it. **(21)**. In parallel to the previous investigation, the current study revealed that rats treated with TMX showed pronounced elevation in the serum liver function enzymes (ALT, AST, and ALP) with concomitant decreases in the serum albumin and total protein **(22)**. The modification in liver function markers was corroborated by significant histopathological degenerative alterations in hepatic tissue of rats subjected to TMX toxicity, potentially indicating hepatocellular damage. **(22).**

The increase in ALT, AST, AST, and ALP in blood circulation may be caused by the leakage of these liver enzymes into the blood stream, which are considered sensitive indicators of liver cell injury in response to TMX toxicity **(23)**. Albumin is the most important protein in blood synthesized by the liver and is a useful indicator of hepatic function **(24).** This study suggests that the decrease in serum albumin (hypoalbuminemia) and total protein could be attributed to defects in protein synthesis caused by liver dysfunction in response to TMX

toxicity. The hepatotoxicity of TMX treatment may relate to its ability to promote oxidative liver damage and generate liver injury with an increase in plasma or serum levels of liver function indicators such as ALT, AST, and ALP **(25).** According to Desai et al. **(22)**, TMX toxicity can lead to the production of reactive oxygen species (ROS). Also another study showed that the oxidation process resulting from TMX intoxication leads to the release of iron ions. These ions become more reactive in the liver. Free iron ions participate in the generation of hydroxyl radicals that can react with the most cellular components and cause cellular tissue damage **(26)**.

Oral ingestion of SLRE to the TMX group markedly ameliorated the deviation in ALT and AST, albumin, and total protein levels versus the TMX untreated group, indicating the protective ability of the plant extract against TMX-induced liver damage. This biochemical result was confirmed by the modulation of histopathological liver picture. The results of our current study agree with Kadhem **(27).** Similarly, it was found that the SLRE can protect the liver from the toxic effect of CCL4 **(28).** The hepatoprotective beneficial impact of SLRE may correlate to its phytochemical constituents. *S. lappa* plant contains active substances, such as saussurine and costunolide lactone, with a wide range of biological activities, including antioxidant and anti-inflammatory properties **(29, 30)**.

In the current study, rats administered TMX for 28 successive days showed a marked elevation in the serum TG and a depletion in T cholesterol, LDL-C, and HDL-C compared to control rats. This effect of tamoxifen on total cholesterol and LDL-C is compatible with the report by Novoa et al. **(31).** Also, clinical studies revealed a significant elevation in circulating TG and reduction in T cholesterol and LDL-C in breast cancer patients after treatment with TMX **(32,33).** In addition, TMX can cause hepatic TG accumulation (fatty liver) in breast cancer patients undergoing TMX chemotherapy . **(34)** reported that hypertriglyceridemia correlated with TMX treatment may be attributed to the reduction in the activity of hepatic triglyceride lipase resulting in impaired clearance of circulating TG. On the other hand, some studies suggested that TMX may act as an estrogen agonist on the liver. This effect plays a key role in altering plasma lipoproteins by (1) increasing very low density lipoprotein synthesis, leading to increased triglyceride levels; (2) decreasing apolipoprotein B synthesis, causing a depletion in LDL cholesterol **(35,36)** .

Treatment of the TMX-intoxicated group with SLRE markedly protected against TMX-induced alterations in serum lipid profiles (T cholesterol and HDL-C). This protection may be attributed to the antidyslipidemic effects of SLRE. These results agree with some investigations reported that SLRE has hypolipidemic effects in diabetic rats **(37,14).** Also, it was found that oral ingestion of *S. lappa*

aqueous extract to rabbits at a dose of 2 mg/kg body weight showed a significant hypolipidaemic effect **(38)**.

The current investigation also revealed a significant increase in serum glucose concentration in rats under the influence of TMX toxicity compared to control ones. This result is consistent with **(39)**, who explained that treatment with TMX can cause liver insulin resistance and reduce liver glucose uptake, leading to the subsequent development of hyperglycemia. Other authors suggested that the severely elevated TG can induce lethal pancreatitis and consequently hyperglycemia **(34)**. In addition, it has been found that treatment with TMX has also been associated with hypertriglyceridemia and steatohepatitis, both of which are features of insulin resistance and glucose intolerance **(40).**

Furthermore, a small study found a significant increase in visceral fat deposition, a prominent feature of insulin resistance, in breast cancer patients treated with TMX compared with controls **(41)**. Preclinical studies indicate that estrogen receptors are present on insulin-producing pancreatic beta-cells, and estrogen appears to protect against beta-cell apoptosis **(42)**. Estrogen inhibition has been associated with increased insulin resistance **(43)** and decreased insulin secretion **(42)**. TMX has been shown to directly increase beta-cell apoptosis and decrease insulin secretion in mice **(42) .**

Intake of SLRE concurrently with TMX administration to rats effectively modulated the alterations in serum glucose level with

respect to TMX-intoxicated rats, which may link to the presence of active substances, such as saussurine and costunolide lactone, in SLRE with antidiabetic useful impact. **(29, 30)**

5 Conclusions

The results of this study lead us to believe that the watery extract of the *Saussurea lappa* root can protect against liver damage caused by TMX, most likely through an antioxidant mechanism. Although studies on SLRE extract are very promising, more research is required to fully evaluate and understand its role in hepatotoxicity prevention.

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Conflict of interest: The author declare that they have no conflicts of interest to disclose .

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