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HEPATOPROTECTIVE POTENTIAL OF SALIX ALBA ON TAMOXIFEN INDUCED HEPATIC STEATOSIS

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is a growing global health concern, and the development of effective and safe therapeutic agents is crucial. The present study aimed to investigate the hepatoprotective potential of Salix alba bark extract (MESA) against tamoxifen-induced hepatic steatosis in rats. MESA was prepared using the Soxhlet extraction method, and its phytochemical constituents were analyzed. Tamoxifen-induced hepatotoxicity was used as the experimental model, and the hepatoprotective effects were evaluated by assessing biochemical parameters, body weight, liver weight, and histopathological changes. MESA at doses of 200 mg/kg and 400 mg/kg significantly attenuated the tamoxifen-induced alterations in serum markers, lipid profile, and histopathological changes in the liver. The findings suggest that MESA exhibits potent hepatoprotective activity, which may be attributed to its antioxidant and anti-inflammatory properties. These results support the potential of Salix alba as a natural remedy for the management of NAFLD.

Index Terms - Salix alba, hepatoprotective, tamoxifen, non-alcoholic fatty liver disease, antioxidant

I. INTRODUCTION

Tamoxifen is a widely used drug in the treatment of various types of cancer, particularly breast cancer. While it has been effective in reducing the risk of cancer recurrence, tamoxifen is also known to cause adverse effects on the liver, leading to the development of a condition called hepatic steatosis[1,2]. Hepatic steatosis is characterized by the abnormal accumulation of lipids, primarily triglycerides, within the liver cells. The development of hepatic steatosis is a significant concern, as it can progress to more severe liver diseases, such as non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis[3,4,5]. Therefore, finding effective strategies to prevent or mitigate tamoxifen-induced hepatic steatosis is of great importance. Oxidative stress and the generation of reactive oxygen species (ROS) play a crucial role in the development and progression of NAFLD[6,7]. The accumulation of free radicals can lead to cellular damage, inflammation, and the activation of hepatic stellate cells, ultimately resulting in liver fibrosis Consequently, therapeutic agents with antioxidant and anti-inflammatory properties may hold promise in the management of NAFLD[8,9]. Salix alba, commonly known as the white willow, is a medicinal plant that has been used in traditional medicine for centuries to treat various liver disorders. The bark of Salix alba is a rich source of bioactive compounds, including salicin, flavonoids, tannins, and phenolic acids, which have been associated with hepatoprotective, antioxidant, and anti-inflammatory activities. However, the potential of Salix alba in the management of NAFLD remains largely unexplored[10,11].

In the present study, we aimed to investigate the hepatoprotective potential of a methanolic extract of Salix alba bark (MESA) against tamoxifen-induced hepatic steatosis in rats. Tamoxifen, a widely used anti-estrogen drug for the treatment of breast cancer, has been reported to induce hepatotoxicity and liver fat accumulation, making it a suitable model for the study of NAFLD. The study evaluated the effects of MESA on biochemical

parameters, body weight, liver weight, and histopathological changes in the tamoxifen-induced hepatic steatosis model[12, 13].

II. MATERIALS AND METHODS

Experimental animals:
Healthy Wistar albino rats, aged 6-8 weeks and weighing 150 ± 10 g, were obtained from the Vidyabharati College of Pharmacy, Amravati, India (CPCSEA Registration no. 1504/PO/RE/S/11/CPCSEA). The animals were housed in a temperature-controlled (22 ± 3°C) environment with a 12-hour light/dark cycle and had free access to standard rodent chow and water. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) and conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Selection of the plant
The medicinal plant Salix alba (Family: Salicaceae) was selected for Hepatoprotective activity based on the literature survey.

Preparation of plant extract
The bark of Salix alba was processed by washing with clean water, air-drying, pulverizing, and sieving through a 0.3 mm sieve. tool consists of several parts including a heat round bottom flask, Soxhlet extractor, and condenser. The solid coarsely powdered bark (500g) was placed in thimble and placed in an extractor. The bottom end of the extractor was connected to a round bottom flask containing a solvent (methanol 1500ml was chosen as the solvent), and was connected to a reflux condenser. The bottom flask was heated to boil the solvent (methanol), the vapor rises through the branch pipe of the extractor, was condensed and drops into the thimble and the solvent (methanol) was contacted with the solid for extraction. When the solvent (methanol) surface exceeds the highest point of the siphon, the solvent containing the extract was return back to the round bottom flask. This cycle was repeated until the all the material extracted from the solid roots powder. The Soxhlet extractor can run continuously without any further operation, making it an excellent choice for extracting compounds over hours or even days. Filtration is not required So it save lot of time, energy and financial inputs. The percentage yield of the extract was calculated and the extract was then subjected to different phytochemical tests.

Solubility analysis
The solubility analysis of MESA of bark has been carried out using different solvent. Extract was found to be soluble in water

Phytochemical screening:
The MESA was screened for the presence of various phytochemicals, including carbohydrates, flavonoids, alkaloids, glycosides, phenolic acids, triterpenoids, sterols, fatty acids, tannins, proteins, and amino acids, using standard qualitative methods.

Drugs and chemicals
Inducing Agent: Tamoxifen 20mg was purchased from the pharmacy store of Amravati, India
Standard drug: Silymarin 70mg tablet manufactured in India by Orion life science and brought from pharmacy store Amravati
Treatment drug: Salix alba bark methanolic extract

Methodology
Thirty male albino wistar rats weighing 200 250 g were selected for the study. Among the 5 groups with 6 animals in each group. Group 1 served as negative control in which the 1ml/kg saline were given through intraperitoneally for 7 days. Group 2 served as positive control in which TAM (45mg/kg/day) given for 10 days. Group 3 served as standard in which rat were treated with silymarin (50mg/kg/day) for 14 days through p.o. Group 4 served as test treatment in which rat were treated with the 1st dose of MESA (200mg/kg) along with TAM for 14 days through p.o. Group 5 served as test treatment in which rat were treated with 2nd dose of MESA (400mg/kg) along with TAM for next 14 days through p.o. After completion of the treatment, animals were anaesthetized under light chloroform anaesthesia. Blood was collected by tail vein puncture. Biochemical estimations of various parameter like body weight, liver weight, cholesterol, triglycerides

(TAG), LDH (Lactate dehydrogenase), serum ALT, ALP, AST and total bilirubin, total protein were assessed. At the end of the protocol animals were sacrificed by cervical dislocation and liver were removed to measure the change in weight and for histopathological evaluation.

Evaluation of pharmacological screening

Tamoxifen induced hepatotoxicity in rat model was assessed for hepatoprotective activity. Following evaluation parameters were assessed for hepatoprotective activity in tamoxifen induced hepatotoxicity rat model.

1. Measurement of body weight

The body weight of the individual rat was recorded on 1st day and after 7th day of intoxicated and at one-week interval till the end of experiment.

2. Liver weight

The liver weight of individual rat was recorded after 7th day intoxicated and sacrificed at the end of experiment.

3. Biochemical Estimation

On the end of experiment all the animals were sacrificed by cervical dislocation and blood sample were collected by tail vein method and serums like ALT, ALP, AST, LDH (Lactate dehydrogenase) Cholesterol, triglycerides (TAG), were observed.

Treatment protocol

Animals (30 Male/female, wistar albino rats), aged 7-8 weeks, weighing (150 ± 10 g) were classified into 5 groups of 6 animals each.

Table 1: Different groups of animals and their treatment.

Sr. no.	Group	No. of Animals	Treatment and Dose	Route of Administration
1	I Normal control	6	Saline treatment (1ml/kg)	IP
2	II Positive control	6	TAM (45 mg/kg/day)	IP/ p.o
3	III Standard	6	TAM (45 mg/kg/day) + SIL (50mg/kg/day)	p.o
4	IV Treatment 1	6	TAM + MESA (200mg/kg)	p.o
5	V Treatment 2	6	TAM + MESA (400mg/kg)	p.o

Statistical analysis

The data obtained from the screenings were subjected to statistical analysis following One-way ANOVA followed by Dunnett Comparison Test to assess the statistical significance of the results using GraphPad prism-9 software.

III. RESULTS

Extraction yield and phytochemical screening:

The extraction yield of the MESA was found to be 37.4% w/w. The phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, phenolic acids, sterols, tannins, proteins, amino acids, phenols, and saponins in the extract.

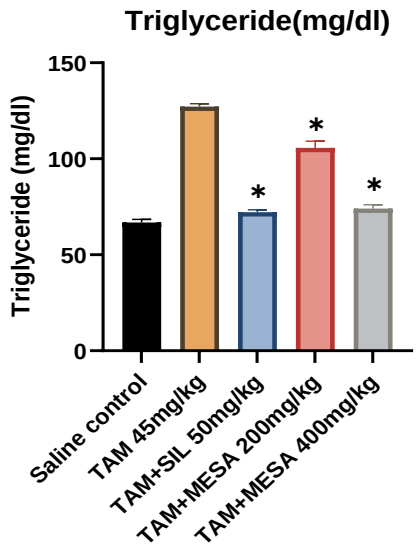
Pharmacological evaluation parameters for hepatoprotective

Table 2: Effect of *Salix alba* on blood parameter in tamoxifen induced model.

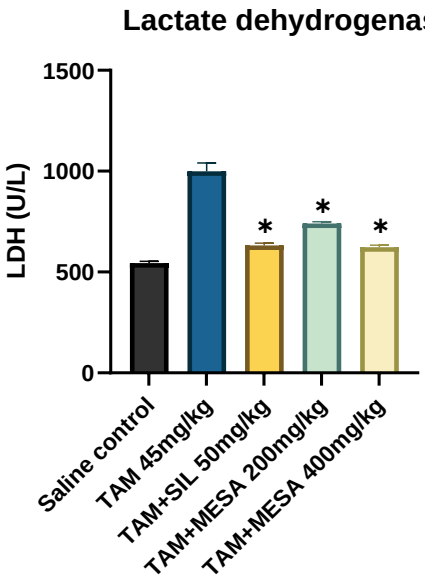
Parameter (n=6)	Saline control (1ml/kg)	TAM (45mg/kg/)	SIL (50mg/kg/)	MESA (200mg/kg)	MESA (400mg/kg)
Body weight	247.7±2.186	260.5±1.522	246.0±2.620*	248.8±2.937*	242.3±2.539*
Liver weight	4.400±0.03651	6.917±0.1447	5.20±0.0361*	5.667±0.033*	5.167±0.033*
Cholesterol (mg/dl)	74.00±1.461	128.2±1.662	71.33±1.430*	106.2±3.637*	68.67±1.282*
TG (mg/dl)	66.83±1.641	127.2±1.537	72.17±1.249*	105.7±3.75*	74.00±2.017*
LDH (U/L)	544.7±8.913	998.8±41.40	632.3±11.27*	741.2±8.635*	623.2±9.669*

All data a, b, c, are expressed as mean ± SEM for (n=6) rat in each group. One-way ANOVA followed by Dunnett's multiple comparisons. Values are statistically significant *P< 0.0001 for Cholesterol, TG, LDH as compared with positive control (TAM 45mg/kg) and saline control

a.



b.



c.

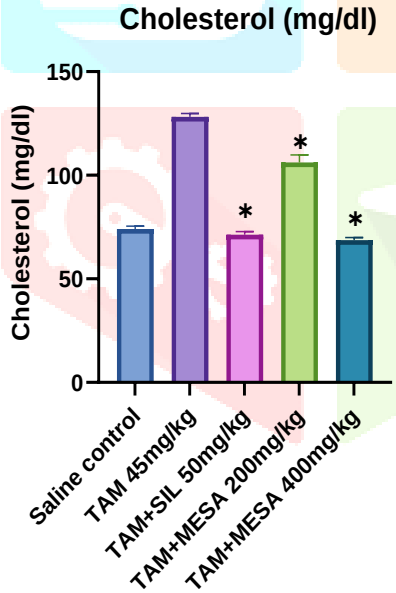


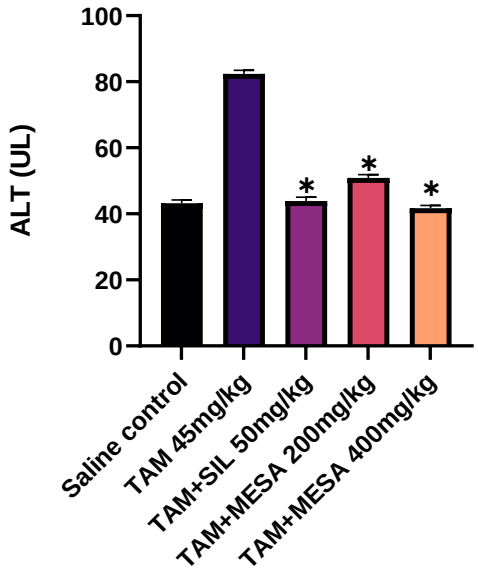
Fig 1: Effect of *Salix alba* on blood parameter in tamoxifen induced model

Table 3: Effect of *Salix alba* on blood parameter in tamoxifen induced model.

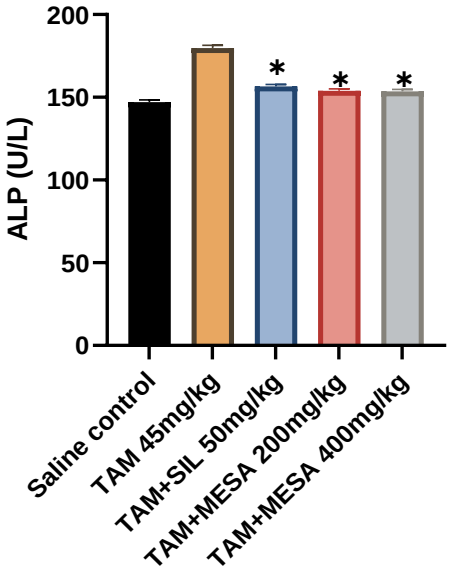
Parameter (n=6)	Saline control (1ml/kg)	TAM (45 mg/kg/)	SIL (50mg/kg/)	MESA (200mg/kg)	MESA (400mg/kg)
ALT(U/L)	43.17 ± 1.078	82.33±1.145	43.83±1.167*	50.83±1.014*	41.67±0.8819 *
AST(U/L)	113.8 ± 3.260	222.5±0.7638	113.8 ± 4.020*	189.8 ± 3.177*	111.2±4.110*
ALP(U/L)	147.0 ± 1.461	179.7 ± 1.820	156.5 ± 1.258*	153.8 ±1.195*	153.5±1.176*
Total bilirubin(mg/dl)	0.2933±0.00843	0.5833±0.01856	0.3217±0.07923 *	0.4933±0.1856 *	0.3467±0.346 *
Total protein(g/dl)	7.333 ± 0.1706	4.700 ± 0.1366	7.683 ± 0.1740*	5.817±0.1424*	6.900±0.1483 *

All data a (ALT), b (ALP), c (AST), d (Total protein), e (Total bilirubin) are expressed as mean ± SEM for (n=6) rat in each group. One-way ANOVA followed by Dunnett's multiple comparisons. Values are statistically significant *P< 0.0001 for ALT, ALP, AST, Total protein, Total bilirubin as compared with positive control (TAM 45mg/kg) and saline control.

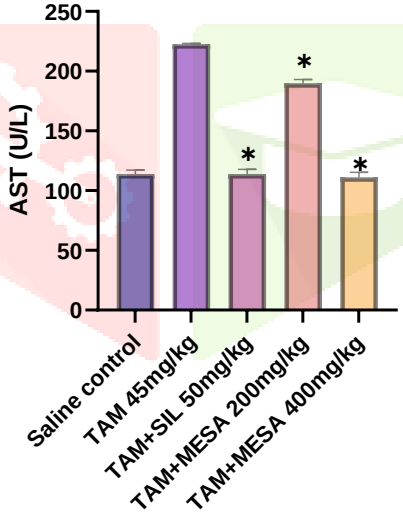
a.


ALT (UL)
100
80
60
40
20
0
*
*
*
Saline control
TAM 45mg/kg
TAM+SIL 50mg/kg
TAM+MESA 200mg/kg
TAM+MESA 400mg/kg

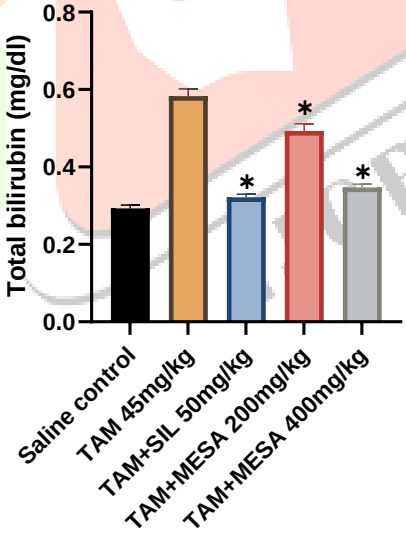
b.


ALP (U/L)
200
150
100
50
0
*
*
*
Saline control
TAM 45mg/kg
TAM+SIL 50mg/kg
TAM+MESA 200mg/kg
TAM+MESA 400mg/kg

c.


AST (U/L)
250
200
150
100
50
0
*
*
*
Saline control
TAM 45mg/kg
TAM+SIL 50mg/kg
TAM+MESA 200mg/kg
TAM+MESA 400mg/kg

d.


Total bilirubin (mg/dl)
0.8
0.6
0.4
0.2
0.0
*
*
*
Saline control
TAM 45mg/kg
TAM+SIL 50mg/kg
TAM+MESA 200mg/kg
TAM+MESA 400mg/kg

e.

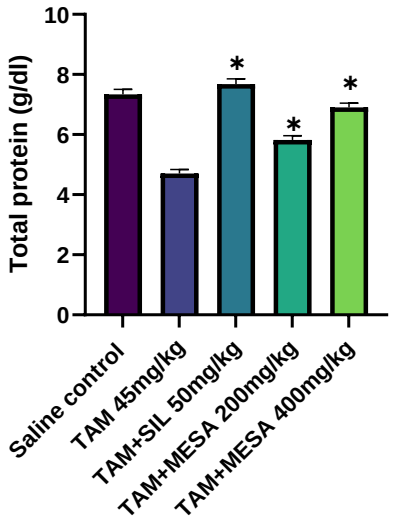


Fig 2: Effect of *Salix alba* on blood parameter in tamoxifen induced model

Tamoxifen being the hepatotoxic agent injure the hepatic cells and cause significant damage to the liver. In this study the total protein level was decreased as compared to the tamoxifen treated rats. The higher concentration of bilirubin and the lower of total protein confirm the depth and intensity of liver necrosis. As a result, from (figure 2) and (table 3) of this ALT, ALP, AST, LDH levels in the groups treated with these hepatotoxic agents were found significantly increased when compared to normal control group in respective studies.

MESA (200mg/kg and 400mg/kg) successfully reduced the level of ALT, ALP, AST, LDH in blood in the treatment group compared to the positive control group. The findings from this study contribute to understanding the potential therapeutic effects of *Salix alba* on various blood parameters associated with hepatic steatosis (NAFLD).

IV. DISCUSSION

The present study evaluated the hepatoprotective potential of Salix alba bark extract (MESA) against tamoxifen-induced liver injury in rats. Tamoxifen, a widely used anti-cancer drug, is known to cause oxidative damage and fatty liver accumulation, making it a suitable model for studying non-alcoholic fatty liver disease (NAFLD). The results showed that MESA treatment effectively attenuated the tamoxifen-induced alterations in biochemical parameters, such as serum levels of liver enzymes (ALT, AST, ALP, LDH), lipid profile (cholesterol, triglycerides), and total protein. These findings suggest that MESA was able to protect the structural integrity of hepatocytes and prevent the leakage of cytoplasmic enzymes into the bloodstream.

The hepatoprotective effect of MESA is likely attributed to its antioxidant and anti-inflammatory properties. The presence of various phytochemicals, including flavonoids, phenolic compounds, and glycosides, in MESA may contribute to its ability to scavenge free radicals and mitigate oxidative stress-induced liver damage caused by tamoxifen administration. The standard hepatoprotective agent, silymarin, was used as a

positive control, and MESA exhibited comparable protective effects, indicating its potential as a natural alternative for the management of NAFLD and other liver disorders. The normalization of serum biochemical parameters and the improvement in histopathological changes observed with MESA treatment support its hepatoprotective potential. In conclusion, the findings of this study provide evidence that Salix alba bark extract possesses potent hepatoprotective activity against tamoxifen-induced liver injury, possibly through its antioxidant and anti-inflammatory mechanisms. These results suggest the therapeutic potential of MESA in the management of NAFLD and warrant further clinical investigations[14].

V. CONCLUSION

Thus, from the present study it can be concluded that the MESA showed potential hepatoprotective effects against chronic liver injury, which is likely due to its antioxidant and anti-inflammatory properties. These effects, at least in part, prevent TAM free radical derivatives formation and hence inhibit cellular damage. The high antioxidant activity of MESA due to its high content of phenolic compounds that possess high radical quenching abilities. Treatment with MESA result in significant reduction in biochemical parameters. Histopathological analysis shows extensive hepatocellular damage, as represented by the presence of portal inflammation, fatty change and venous congestion. Accordingly, our findings may play a role towards the discovery of a new naturopathic remedy.

Future Prospective

Salix alba, the white willow, exhibits promising potential in the management of non-alcoholic fatty liver disease (NAFLD) due to its multifaceted mechanisms of action. The bark of Salix alba contains salicin, which can be metabolized to salicylic acid, an active compound with potent antioxidant and anti-inflammatory properties. These effects can help mitigate the oxidative stress and inflammatory processes that contribute to the development and progression of NAFLD. Additionally, studies have shown that Salix alba extracts can regulate lipid metabolism, reducing the accumulation of fat in the liver. As the burden of NAFLD continues to grow, the therapeutic potential of Salix alba warrants further exploration as a natural, plant-based approach to manage this condition.

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