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RESEARCH ARTICLE

Evaluation of hepatoprotective and anticancer potential of *Ehretia Laevis*: An *In-vitro* evidence

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ABSTRACT:
Background: *Ehretia laevis* has been used to treat Jaundice in various Uttarakhand sub-Himalayan regions in India. The current study focused primarily on *Ehretia laevis* hepatoprotective and anticancer activity. **Objective:** The present study validates *Ehretia Laevis* traditional claim as a jaundice remedy by evaluating *in vitro* hepatoprotective properties of *Ehretia laevis* leaves along with their anti - cancer properties. **Materials and Methods:** The well-known model of carbon tetrachloride induced in vitro rat hepatocyte injury in HepG2 cells, the hepatoprotective effect of the hydroalcoholic extract, ethyl acetate fraction and aqueous fraction of *Ehretia laevis* was evaluated. Further the concentrations of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), triglycerides (TGL) and total proteins in the culture medium were evaluated as an indication of hepatocytes necrosis using Ecoline diagnostic kits. The MTT assay was used to estimate the *in vitro* anticancer activities of the hydroalcoholic extract, ethyl acetate fraction and aqueous fraction of *Ehretia laevis* against cancer cell lines [Vero (African, Green Monkey Kidney), MCF-7 (Human, Breast Carcinoma), A-549 (Human, Lung Carcinoma) HEP-G2 (Human, Liver Carcinoma)]. **Result:** Among the extracts evaluated ethyl acetate fraction showed significant Hepatoprotective and anticancer activities at low concentration. *Ehretia laevis* demonstrated dose-dependent hepatoprotective activity. **Conclusion:** The present study validates its traditional use as a remedy of jaundice and can provide promising therapeutic interventions against chemical-induced liver damage. Moreover *Ehretia laevis* also showed significant anti-cancer activity.

KEYWORDS: Anticancer, Ehretia Laevis, Hepatoprotective, MTT, Cell line

INTRODUCTION:
Despite huge advances in present day medication no viable medications are accessible to animate liver capacities and offer security to the liver from the harm or help to recover hepatic cells. Without solid liver-defensive medications in present day drug, a substantial number of home grown arrangements are prescribed for the treatment of liver issue and regularly professed to offer huge alleviation. Endeavors are being made all around to get logical confirmations for these customarily revealed home grown medications[1].

Ehretia laevis is an imperative therapeutic plant. Every single segment of this plant is utilized for various therapeutic purposes. In certain pieces of India, a decoction of the crisp root is utilized for the treatment of syphilis[2], while a decoction of the stem bark is utilized inside and as a swish in throat infections. *Ehretia laevis* has for some time been utilized for treating Jaundice in different Sub-Himalayan locale of Uttarakhand in India[3]. The plants of the class have critical therapeutic significance and discover utilizes in conventional medication as a solution for the treatment of diarrhea, cough, cachexia, syphilis, hostile to arthritic, against platelet, calming activities, and in osteoarthritis[4,5]. The comparison of antioxidant activity of stem and leaves of ehretia laevis carried out [6].

It has moreover been represented to have moderating and threatening to bacterial activities. At any rate this plant has still not been evaluated for its hepatoprotective and

anticancer activities. The purpose of the present examination is to affirm traditional case the of *Ehretia laevis* as fix of jaundice by evaluating *in-vitro* hepatoprotective properties of blooms of *Ehretia laevis* close by its anticancer properties. In any case, there are just a couple of logical examinations on hepatoprotective action in the writing. Consequently, the present examination was wanted to assess the anti-oxidant and hepatoprotective activity of hydroalcoholic (70%) concentrate and ethyl acetic acid derivation part of *Ehretia laevis* Rox bin.

MATERIAL AND METHODS:

Chemicals:

Vero (African, Green Monkey Kidney), MCF-7 (Human, Breast Carcinoma) and A-549 (Human, Lung Carcinoma) HEP-G2 (Human, Liver Carcinoma) cell lines were procured from the National Centre for Cell Sciences, Pune, India. MTT (3-(4, 5 dimethyl thiazole-2 yl) - 2, 5-diphenyl tetrazolium bromide), purchased from Sigma (St Louis, MO, USA). Standard silymarin, aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline Phosphatase (ALP), triglycerides (TGL) Ecoline diagnostic kits were used.

Plant material:

Flowers of *Ehretia laevis* plants, collected during the month of February 2018 from the Ambajogai, district Beed of Maharashtra state and was authenticated from Botanical Survey of India [Authentication number: No. BSI/WRC/100-2/Tech./2018/37], Ministry of Environment, Forest and Climate Change, Western regional center, Pune (Maharashtra) India.

Preparation of Hydroalcoholic Extract and Fractions:

The blooms *Ehretia laevis* were dried at room temperature for fifteen days and afterward decreased to a coarse powder. The plant powder was extricated with ethanol (70%) for 12 h at 50 ˚0 C with Hot Continuous Extraction (Soxhlet) technique. The got concentrate was focused under diminished weight on turning evaporator at 40 ºC to get a tanish buildup. The above concentrate was broken up in refined water and divided successively with n-hexane, ethyl acetic acid derivation and watery to get n-hexane, ethyl acetic acid derivation and fluid divisions. Every one of these portions was concentrated utilizing rotating evaporator[7-12].

Determination of mitochondrial synthesis by MTT assay:

The monolayer cell culture was trypsin zed and the cell check was changed in accordance with 1.0x105 cells/ml utilizing DMEM medium containing 10% FBS. To each well of a 96 well microtiter plate, 100µl of the weakened cell suspension (around 10,000 cells/well) was included. Following 24 hours, when an incomplete monolayer was framed, the supernatant was flicked off; the monolayer was washed once with medium and 100 µl of various test focuses arranged in support media were added per well to the halfway monolayer in microtiter plates. The plates were then hatched at 37 ˚C for 48 hrs in 5% CO2 climate, and minuscule examination was done and perceptions recorded like clockwork. Following 48 hours, the example arrangements in the wells were disposed of and 20 µl of MTT (2mg/ml) in MEM-PR (MEM without phenol red) was added to each well. The plates were shaken and hatched in 5% CO2 environment for 3 hours at 37oC. The supernatant was evacuated and 50 µl of iso-propanol was included and the plates were delicately shaken to solubilize the shaped formazan. The absorbance was estimated utilizing a microplate reader at a wavelength of 540 nm. The rate development restraint was determined utilizing the accompanying equation and convergence of medication or test tests expected to hinder cell development by half qualities were created from the portion reaction bends for every cell line[13-19].

$$\% \text{ Cell Viability} = \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Carbon tetrachloride induced *in vitro* hepatocytes injury:

CCl_4 instigated hepatocytes damage was completed after a brooding of 24 h. the hepatocytes were presented to the new medium containing CCl_4 (1%) alongside/without different groupings of the test tests or the medium alone (as should be expected). Centralizations of the test tests and standard silymarin more noteworthy than 250 µg/ml were observed to be poisonous to the cells. Following an hour of CCl_4 challenge, convergences of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline Phosphatase (ALP), triglycerides (TGL) and all out proteins in the medium utilizing Ecoline analytic packs, were estimated for the sign of hepatocytes rot[20-24].

Statistical analysis:

Results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using *Dunnett's* multiple comparison tests and one-way analysis of variance (ANOVA) using GraphPad Prism software, USA. Results were considered statistically significant at $P<0.05$ and $P<0.01$.

RESULTS:

Extraction:

The blooms *Ehretia laevis* were dried and separated with ethanol (70%), hexane, ethyl acetic acid derivation and water by Soxhlet mechanical assembly. The most extreme yield was gotten with ethanol pursued by water, ethyl acetic acid derivation and hexane separately. The

extractive yields of the concentrate are mentioned in table 1. The ethanolic(70%) concentrate and ethyl acetic acid derivation division were exposed to subjective phytochemical investigation which uncovered the nearness of phenolics, flavonoids, tannins, saponins, terpenoids, sterols, starches and glycosides.

Table no. 1: Yields of different extracts

Sr. No.	Extract	Yield
1	70% Ethanolic extract	23.25%
2	Hexane fraction	0.75%
3	Ethyl acetate fraction	9.50%
4	Aqueous eraction	15.35%

In-vitro hepatoprotective activity:

The after effects of the *in vitro* hepatoprotective activity of the hydroalcoholic concentrate and its parts are given in the Table 2. The hydroalcoholic concentrate and its divisions at the tried centralizations of 125 and 62.5, 32.25 and 15.625 µg/ml demonstrated a portion subordinate security against CCl_4 incited rise in the AST, ALT and ALP. However, just hydroalcoholic separate at the tried convergences of 125 and 62.5 demonstrated noteworthy portion subordinate insurance against CCl_4 initiated rise in the AST, ALT and ALP ($P<0.05$). Standard, silymarin at convergence of 250 µg/ml fundamentally kept the CCl_4 prompted height in the AST, ALT and ALP ($P<0.05$). Moreover the hydroalcoholic extricate at the tried fixations demonstrated noteworthy impact on TP and TGL levels ($P<0.05$). Additionally standard Silymarin at centralization of 250 µg/ml indicated critical impact on TP and TGL levels ($P<0.05$). (table 2)

Table no. 2: Effects of *Ehretia laevis* extracts and fractions on biochemical parameters in CCl_4 intoxicated freshly isolated Rat hepatocytes.

	Concentration	AST U/L	ALT U/L	ALP U/L	TP g/dL	TGL mg/dL
Normal	-	17.00	13.00	32.00	1.5	156
CCl_4	1%	85.00	56.00	100.00	0.7	70.00
CCl_4 (1%) + Silymarin	250 µg/ml	18.00	14.00	35.00	1.21	150
CCl_4 (1%) + Sample No. 1	250	--	--	--	7.63	51.9
	125	57.70	72.98	8.25	10.29	41.5
	62.5	66.40	74.72	19.20	8.65	42.50
	31.25	65.44	61.28	32.70	10.77	47.30
	15.625	79.36	59.36	27.70	8.11	67.20
CCl_4 (1%) + Sample No. 2	250	--	--	--	10.06	64.6
	125	19.36	68.40	40.25	8.25	66.3
	62.5	44.58	61.20	34.00	7.70	23.3
	31.25	97.90	30.80	33.70	8.52	28.59
	15.625	92.05	39.60	31.70	8.59	22.60
CCl_4 (1%) + Sample No. 3	250	--	--	--	12.13	121.00
	125	20.53	15.07	38.70	8.59	93.80
	62.5	38.41	90.00	80.25	7.97	71.30
	31.25	47.70	84.40	85.20	7.43	76.60
	15.625	175.50	80.70	88.70	8.45	62.80

Table no. 3: Effect of *Ehretia laevis* on cytotoxicity using MTT assay

Sample No.	Sample description	Vero IC_{50} µg/ml	MCF-7 IC_{50} µg/ml	A-549 IC_{50} µg/ml	HEP-G2 IC_{50} µg/ml
1	EA	123.2809	82.248	55.01493	87.12588
2	Aq	292.6872	142.8434	124.8835	228.0151
3	70% Alcohol	289.0694	143.702	88.24742	87.12588
4	5-FU	123	12.61	8.191	14.901

Cytotoxicity of EA, Aq, 70% alcohol and 5- FU against Vero, MCF-7, A-549 and HEP-G2 cell line, a MTT cell viability assay

In-vitro anticancer activity:

The cytotoxic activity of the concentrates *Ehretia laevis* on Vero (African, Green Monkey Kidney), MCF-7 (Human, Breast Carcinoma) and A-549 (Human, Lung Carcinoma) HEP-G2 (Human, Liver Carcinoma) MCF was examined *in vitro* using 3-(4) 5 Dimethyl-thiazol-Zyl) 2,5 biphenyl tetrazolium bromide (MTT). The outcomes indicated diminished cell feasibility and cell development in a portion subordinate way. Ethyl acetic acid derivation portion and 70 % ethanolic remove showed solid enemy of proliferative exercises. Since the phytochemical investigation has demonstrated the nearness of intense phytochemicals like, phenols, flavonoids, terpenoids, Saponins, steroids, and so on. A few creators have revealed that phenolic, flavonoids, steroids, terpenoids are known to be bioactive standards. The cytotoxicity contemplates as appeared in table 3.

DISCUSSION:

In the present investigation, we have described the hepatoprotective and hostile to malignant growth action of *Ehretia laevis*. So as to comprehend the normal for the cytotoxicity impact of Ehretia laevis extricate on malignant growth cells, two cells lines were chosen to be examined all through this examination; the carcinogenic MCF-7 cell lines and the control serving non-harmful Vero cell lines. The present examination additionally showed the cytotoxicity records as a proportion of rate

cell mortality determined by MTT measure in MCF-7 and Vero cells separately, in a portion subordinate way toward the finish of 24 hours brooding with concentrate. Bosom malignant growth cell line MCF-7 was utilized as the test framework in this examination which was incited by the necessity of progressively compelling treatment for the expanding rate of bosom diseases around the world. The concentrate had the capacity to repress the expansion of the disease cell and the ordinary Vero cells. The American National Cancer Institute (NCI) rules set the cutoff of action for unrefined concentrates at half restraint (IC50) of multiplication of under 30 µg/mL after the introduction time of 72 hours[25]. Anyway an unrefined concentrate with IC50 under 20 µg/mL is considered very cytotoxic [26]. The consequences of the present examination indicated intense cytotoxic impacts on MCF-7 cells with Ehretia laevis extricate. The IC50 esteem was observed to be lower than that predetermined by NCI, USA for arrangement of an unadulterated compound as anticancer specialist. The decrease in practical cell number was apparent as 24 hours of treatment with both the concentrates and divisions. The morphological impacts were increasingly unmistakable in the portions treated cells demonstrating broad blebbing and vacuolation recommending autophagic component of cell demise.

Preliminary phytochemical results confirmed the presence of flavonoids, saponins, steroids and tannins in the extracts of *Ehretia laevis* [27]. Steroids and tannins are hepatoprotectives[28-29]. It is also proved that saponins hold a lot of therapeutic potential like antidiabetic, anti-inflammatory, anticancer, antimicrobial, cardioprotective [30]. In the appraisal of CCL4-interceded hepatotoxicity, the assurance of marker protein, for example, AST, ALT, ALP and TP is to a great extent utilized. In the present examination, a huge ascent in the dimension of AST, ALT, ALP and TP in CCL4 treated cells was watched, demonstrated the expanded porousness of hepatocytes and impressive cell harm. Treatment with Ehretia laevis concentrates and parts essentially diminished these chemical exercises, showing that Ehretia laevis could keep up the useful uprightness of hepatocyte layer, in this manner securing the hepatocytes against CCL4 poisonous quality. In rodent hepatocyte culture, Ehretia laevis was additionally observed to be powerful in diminishing the spillage of ALT and AST activated by CCl4, demonstrating in vitro hepatoprotective movement.

CONCLUSION:

The selected plant *Ehretia Laevis extracts* shown good hepatoprotective and anticancer activities. Hence the study can further be taken up to explore these plants in depth.

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