ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Antioxidant and Hepatoprotective activity of *Ehretia laevis Roxb* against paracetamol induced acute Hepatotoxicityin wistar rats

Tarke Santosh Rangnathrao[1]*, P. Shanmugasundaram[2]
[1]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, VELS Institute of Science, Technology and Advances Studies (VISTAS), Chennai-600117
[2]Director, VELS College of Pharmacy, VELS University, Chennai -600117
*Corresponding Author E-mail: **tarkesantosh@gmail.com**

ABSTRACT:
The aim of the current study is to evaluate the antioxidant activity and hepatoprotective activity of hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis Roxb* in wistar rats against paracetamol-induced acute hepatotoxicity. In addition, reducing the power of the extracts and their ability to scavenge free radicals were evaluated by applying 2, 2-diphenyl 2-picrylhydrazyl hydrate (DPPH) and Reducing Power Assay. The hydroalocoholic (70%) extract and its ethyl acetate fraction of *Ehretia laevis* displayed strong antioxidant activity. The extracts at a dose of 100 and 200 mg/kg, p.o., showed significant dose-dependent protection against paracetamol-induced changes in the serum ASAT, ALAT, ALP, and TP. The hydroalocoholic (70%) extract and its ethyl acetate fraction also showed dose-dependent protection against Paracetamol-induced changes in liver tissue such as fatty degeneration, lymphatic infiltration, and necrosis. Among the extracts tested the ethyl acetate fraction shows better activity than the Hydroalocoholic extract.

KEYWORDS: Antioxidant, DPPH, *Ehretia laevis* Roxb, Hepatoprotective, Nitric Oxide.

INTRODUCTION:
The liver, being the center of metabolic functions, plays a crucial role in metabolizing a variety of xenobiotics, viral infections, and chronic alcoholism[1]. It is involved in almost all the biochemical pathways related to growth, to fight against the disease, nutrient supply, energy provision, and reproduction[2]. Hepatotoxicity is one of the very common ailment resulting into serious debility even mortality. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damage[3]. Toxic chemicals (e.g. peroxidized oil, carbon tetrachloride, chlorinated hydrocarbons etc.), several drugs (paracetamol, isoniazid, certain antibiotics, chemotherapeutics etc.), viral infections, autoimmune challenges and excess alcohol consumption are known to cause liver diseases. Liver disease is still a serious health problem affecting more people worldwide at an alarming rate[4]. Most common drugs involved in drug-induced liver injury (DILI) are non-steroidal anti-inflammatory drugs, anti-tuberculosis drugs, antibiotics, anticonvulsants, anesthetics and herbs[5].

Received on 31.05.2019 Modified on 25.06.2019
Accepted on 22.07.2019
Research J. Pharm. and Tech. 2019; 12(12): 6143-6148.
DOI: 10.5958/0974-360X.2019.01067.9

The real incidence of DILI remains unknown due to difficulty in establishing a diagnosis and the subclinical nature of injury in many cases. Its annual incidence in general population ranges from 14 to 19 per 100,000 inhabitants, with approximately 30% exhibiting jaundice. Overall mortality from 10 to 17.3% has been observed in several series[6]. Paracetamol (Acetaminophen) is an analgesic, antipyretic drug and metabolized by cytochrome P450 system, which leads to the formation of N-acetyl-p-benzoquinoneimine (NAPQI), which causes oxidative stress and depletion of glutathione[7,8]. In the present time, many newly developed drugs (e.g., Rimonabant, Propylthiouracil, or Corticosteroids) have been used for the treatment of liver diseases; however, these drugs possess harmful side effects such as insomnia, vomiting, constipation, and depression. For that reason, nowadays, studies are extensively exploring natural products to maintain liver function and treat diseases of the liver.

Ehretia laevis Roxb is one of such plants that are being used to treat jaundice in a various sub-Himalayan region of Uttarakhand. *Ehretia laevis*, an Indian medicinal plant, is a deciduous shrub considered to be a small tree due to its height (12m). Predominant chemicals from this plant are Naphthoquinone derivative named lewisone, n-

octatricontane, baurenol acetate, baurenol, ursolic acid, Amino acids, Proteins, Lipids, Minerals like Ca, Na, NH_3, Mg, Fe, Mn, K, P, Zn, Cu and Si, Total phenolics content in leaves, Tannins in stem bark, flavonoids and Vitamin C in fruits, leaves and fruits showed the presence of acontanes, decanoic acids, phthalic acid, phytol, a and ß amyrin, piperazine, phenylephrine, naphthoquinone lewisone, bauerenol, bauerenol acetate, a-amyrin, betulin, lupeol, betulinic acid, ß-sitosterol. dodecane, tridecene, tetradecane, n octylcyclohexane, tridecanol, hexadecane, decylcyclohexane, heptadecane, nonadecane, tetratetracontane, amino acid- butyric acid, ornithine, cysteine, histidine, arginine, serine, hydroxy proline, glutamic acid, proline, lysine, tryptamine having various therapeutic properties[9]. As mentioned in Ayurveda, *E. laevis* Roxb, belonging to family Boraginaceae, is an important medicinal plant. The plants of the genus have significant medicinal importance and find uses in traditional medicine as a remedy for the treatment of diarrhea[10], cough[10], cachexia[10], syphilis[10], anti-arthritic[11], anti-platelet[11], anti-inflammatory activities[11], and in Osteoarthritis[12]. However, there are only a few scientific studies on hepatoprotective activity in the literature. Hence, the present study was planned to evaluate the antioxidantand hepatoprotective activity of hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis Roxb*in Wistar rats against paracetamol-induced acute hepatotoxicity.

MATERIAL AND METHODS:

Chemicals:

Biochemical kits were purchased from Merck Chemicals Private Ltd. India. All the chemicals and solvents used for this activity were of analytical grade.

Plant Material:

The flowers of *Ehretia laevis* plants were collected during the month of February 2018 from the Ambajogai, district of Maharashtra. The plant was authenticated by 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.

Extraction procedure[13-17]:

The plant material of *Ehretia laevis* was dried at room temperature for fifteen days and then reduced to a coarse powder. The plant powder was extracted with 70 % hydroalocoholic for 12 h at 50^0C. The obtained extract was concentrated under reduced pressure on a rotary evaporator at 40ºC to obtain a brownish residue. The above extract was dissolved in distilled water and partitioned sequentially with N-hexane, ethyl acetate and water to obtain N-hexane, ethyl acetate and aqueous fractions. All these fractions were concentrated using rotary evaporator.

DPPH radical-scavenging activity[18-22]:

Radical scavenging activity of plant extracts against stable 2, 2-diphenyl 2-picrylhydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams et al. 1995. One milliliter of the extract or standard antioxidant ascorbic acid at different concentrations was added to 0.5 mL of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (mg/mL), the antiradical dose required to cause a 50% inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

Percentage inhibition = 100 - [A_{sample} / $A_{control}$ x 100]

A_{sample}= absorbance of the sample,

$A_{control}$= absorbance of the control

Nitric Oxide Radical Scavenging Assay[23-27]:

Nitric oxide scavenging activity can be estimated by the slightly modified method of Griess IlIosvoy reaction (Garrat, 1964). 1.0 ml Sodium nitroprusside (5mM) in 20mM phosphate-buffered saline (PBS) pH 7.4 was mixed with 1.0 ml of different concentrations of a test sample and incubated at 25°C for 150 min. The 0.5ml of the above solution was later reacted with 1ml of Greiss reagent. The absorbance of the chromophore formed during the diazotization of nitrites with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm.

$$\text{NO Scavenged (\%)} = \frac{(A_{control} - A_{test})}{A_{control}} \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the test sample.

Animals:

Animal studies were performed as per rules and regulations in accordance to guideline of CPCSEA with registration number JSSCP/OT/IAEC/06/2018-19 dated: 30.04.2018.

Acute toxicity study[28, 29]:

Acute oral toxicity was performed as per Organization for Economic cooperation for development (OECD) guideline 423 methods. Female non-pregnant rats animals were weighed and test substance was administered orally through gavage using specially designed rat oral needle. After the administration of test substance, food was withheld for 2 hrs, but not water. Animals were observed individually after at least once during the first 30 minutes, periodically during the first

24hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days.

Hepatoprotective activity[30-34]:
Thirty-six male Wistar rats were randomized into 6 groups of six each.

Group 1 received 0.5% Carboxymethyl cellulose (CMC), at a dose of 10 ml/kg, p.o. and served as normal control
Group 2 received 0.5% CMC, at a dose of 10 ml/kg, p.o. and served as negative control groups
Group 3 received hydroalocoholic extract at a dose of 100 mg/kg, p.o.
Group 4 received hydroalocoholic extract at a dose of 200 mg/kg, p.o.
Group 5received ethyl acetate fraction at a dose of 100 mg/kg, p.o.
Group 6 received ethyl acetate fraction at a dose of 200 mg/kg, p.o.

All the animals were given the assigned treatment for a period of 14 days. On day 14, three hours after the last treatment all the groups, except Group 1 were administered paracetamol (2.5 g/kg, p.o.) suspension. 48 hours after paracetamol administration, the animals were anesthetized using diethyl ether, and the blood was collected from retro-orbital plexus for biochemical estimations. The animals were then sacrificed by cervical dislocation, and the liver was removed for histopathological analysis.

Biochemical estimation:
The levels of serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP) and total proteins (TP) were analyzed using commercial kits to assess the acute hepatic damage caused by paracetamol.

Histopathological studies:
The liver tissue was collected and immediately fixed in 10% formalin, dehydrated in hydroalocoholic (50–100%), cleared in xylene and embedded in paraffin. Sections (4–5 µm) were prepared and then stained with hematoxylin and eosin (H&E) dye for photomicroscopic observations.

Statistical analysis:
The data are represented as mean ± SD. Results were analyzed statistically by one-way ANOVA followed by Dunnett's multiple comparison tests using Prism software (Version 8). The minimum level of significance was set at $p < 0.05$.

RESULTS:

Acute Toxicity Study:
The extracts of *Ehretia laevis* did not produce any significant morbidity and mortality with doses upto 2000mg/kg. Based on the acute oral toxicity study, the doses of *Ehretia laevis* were fixed at 100mg/kg ($1/20^{th}$ of LD_{50}) and 200mg/kg, p.o., ($1/10^{th}$ of LD_{50}) for the evaluation of the hepatoprotective property.

Antioxidant studies:
DPPH radical-scavenging activity:
The antioxidant potential of aqueous fraction, hydroalocoholic and ethyl acetate fraction was evaluated by DPPH method. Among the three extracts, the hydroalocoholic extract of *Ehretia laevis* showed maximum antioxidant potential with an IC50 value of 56.50µg/ml followed by aqueous fraction (IC50 239.72µg/ml) and ethyl acetate fraction (IC50 350.85µg/ml) as summarized in Table 1.

Nitric Oxide Radical Scavenging Assay:
The antioxidant potential of aqueous fraction, hydroalocoholic and ethyl acetate fraction was evaluated by nitric oxide radical scavenging assay method. Among the three extracts, the hydroalocoholic extract of *Ehretia laevis* showed maximum antioxidant potential with an IC50 value of 478.76µg/ml followed by aqueous fraction (IC50 659.87µg/ml) and ethyl acetate fraction (IC50 892.43µg/ml) as summarised inTable 1.

Table 01: DPPH and Nitric Oxide radical-scavenging activity

S. No	Sample	DPPH radical-scavenging activity IC_{50}(µg/ml)	Nitric Oxide Radical Scavenging Assay IC_{50}(µg/ml)
1	Aqueous fraction	239.72	659.87
2	Hydroalocoholic extract	56.50	478.76
3	Ethyl acetate fraction	350.85	892.43
4	Ascorbic acid	7.77	-
5	Quercitin	-	33.68

Hepatoprotective activity:
The hepatoprotective activity of Hydroalocoholic extract and ethyl acetate fraction of *Ehretia laevis* was evaluated by paracetamol-induced hepatotoxicity model. No significant change in body weight was observed in any group during the entire experimental period. The levels of ASAT, ALAT, ALP, and TP were significantly increased in the negative control group, which confirms hepatotoxicity caused by administration of paracetamol. The treatment with hydroalocoholic extract and ethyl acetate fraction of *Ehretia laevis* led to significant restoration of elevated ASAT, ALAT, ALP, and TP levels. Both the Hydroalocoholic extract and ethyl acetate fraction of *Ehretia laevis* showed a dose-dependent hepatoprotective action in paracetamol-

induced hepatoprotective model. The ethyl acetate fraction of *Ehretia laevis* at 200mg/kg was found to be the most effective among the four test samples in a restoration of normal liver function (as summarised in Table 2).

Table 02: Effect of Hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis* Roxb on body weight and serum biochemistry in rats treated with Paracetamol

	Body Weight in g	**Serum ASAT (U/L)**	**Serum ALAT (U/L)**	**Serum ALP (U/L)**	Serum Total Proteins (g/dl)
Normal Control	245.83±8.80	86.08±2.54	59.60±1.18	145.88±3.73	12.23±0.61
Negative Control	241.82±7.55	132.87±6.97^{++++}	107.27±3.24^{++++}	213.80±6.51^{++++}	8.47±0.37^{++++}
Hydroalocoholic extract100 mg/kg	248.42±5.27	110.68±3.62*	81.02±1.87****	168.40±4.18****	10.33±0.38*
Hydroalocoholic extract 200 mg/kg	246.78±7.53	111.22±3.60*	69.03±1.26****	158.30±5.23****	11.47±0.52***
Ethyl acetate fract. 100 mg/kg	249.48±8.07	106.17±4.28**	70.98±2.29****	161.87±5.38****	11.45±0.46***
Ethyl acetate fract. 200 mg/kg	254.53±6.81	99.40±4.38***	54.78±1.66****	142.37±5.06****	12.27±0.30****

Values are mean ± SD, n=6. The negative control group was compared with normal control and the test groups were compared with negative control group.

$^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001, $^{++++}$p<0.0001 when compared to Normal control, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 when compared to Negative control.

Effect on liver histopathology:

The histopathological analysis of liver tissue of animals treated with Hydroalocoholic extract and ethyl acetate fraction at a dose of 100 and 200 mg/kg, p.o., showed a dose-dependent protection against Paracetamol induced changes such as, fatty degeneration, lymphatic infiltration, and necrosis. The protection was higher in the groups that were treated with 200mg/kg of hydroalocoholic extract and ethyl acetate fraction. The liver of the animals treated with 200mg/kg of hydroalocoholic extract and ethyl acetate fraction offered significant hepatoprotection with near-normal appearance of the hepatic parenchyma including the portal areas (Figure 03).

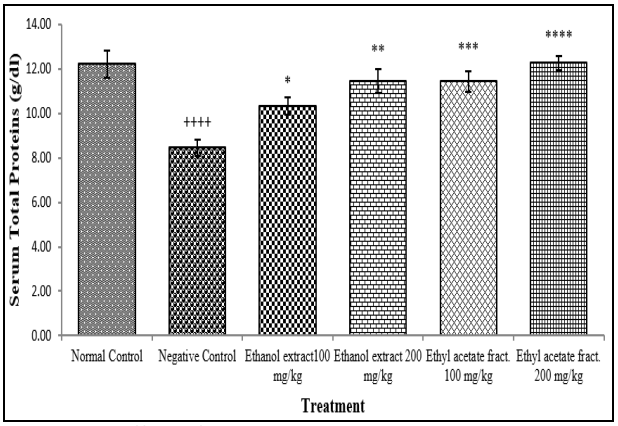


Figure 02: Effect of Hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis Roxb* on serum total protein (g/dl) in rats treated with Paracetamol

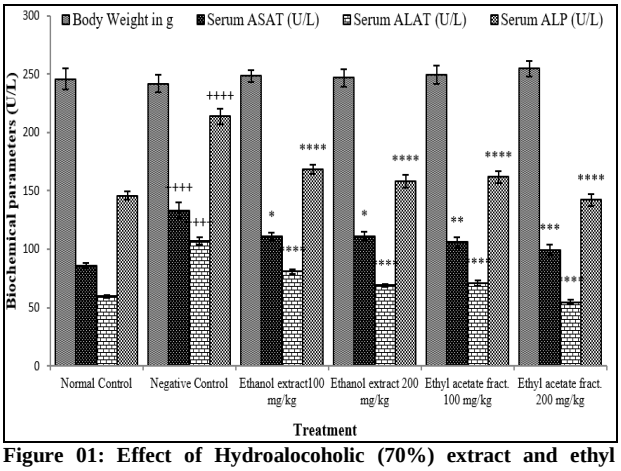
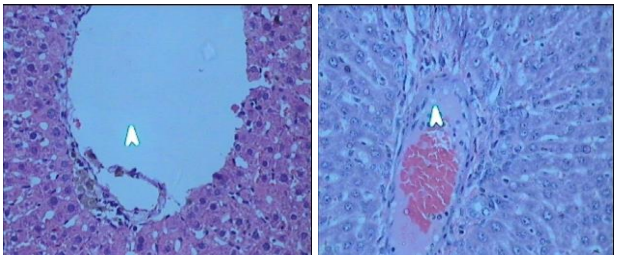
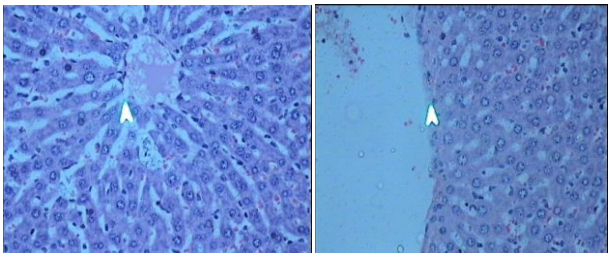


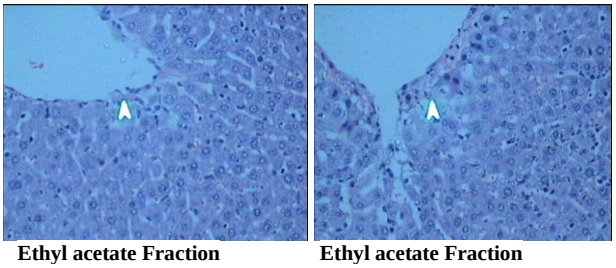
Figure 01: Effect of Hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis* Roxb on body weight and serum biochemistry ASAT, ALAT, and ALP in rats treated with Paracetamol



Normal **Control**



Hydroalocoholic Extract 100mg/kg **Hydroalocoholic Extract 100mg/kg**



Ethyl acetate Fraction 100mg/kg **Ethyl acetate Fraction 100mg/kg**

Figure 03: The histopathological analysis of liver tissue of animals treated with Hydroalocoholic extract and ethyl acetate fraction of *Ehretia laevis Roxb*

DISCUSSION:

The liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds. Any injury or impairment of its function may lead to several implications on health. Management of liver diseases is still a challenge to modern medicine. Conventional drugs used in the treatment of liver diseases are often inadequate. It is necessary to search Alternative drugs for the treatment of liver diseases and to replace the currently used drugs of doubtful efficacy and safety. In this research, 100 and 200mg/kg hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis* Rox was administered to the rats in the test group in order to investigate its protective potential on PCM-induced hepatotoxicity and antioxidant activity in rats.

The model DPPH free radical scavenging assay is an easy method to evaluate antioxidant activity in a relatively short time compared to the other methods. DPPH is a relatively stable radical[35]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. The antioxidant activity of plant extracts was calculated according to the percentage inhibition in DPPH assay. hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis Roxb*, exhibited strong activity on scavenging DPPH radicals with the determined IC50 values are 56.50 and 350.85. Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, and inhibition of platelet aggregation and regulation of cell-mediated toxicity. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions[23]. Both extracts exhibited strong activity on scavenging Nitric oxide radicals with the determined IC50 values are 478.76 and 892.43. The DPPH and Nitric oxide free radical scavenging activity of the plant extracts are shown in Table 01. Thus extracts exhibited significant antioxidant property in both DPPH assay and nitric oxide free radical scavenging assay. The antioxidant activity of the extracts might be due to the presence of phenols and flavonoids. It is suggested that flavonoids and phenols mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions which aid in the hepatoprotective activity of the hydroalocoholic and ethyl acetate fraction of *Ehretia laevis*.

Hepatotoxicity is the potential complication of paracetamol, which is wide, used in general medicine and an assessment of its relative toxicity is important. Hepatotoxicity due to paracetamol overdose causes an excess of glucuronidation and sulfation with the formation of excess NAPQI via cytochrome P450 2E12. NAPQI is detoxified at the expense of reduced glutathione (GSH). Thus, the overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction and the development of acute hepatic necrosis[4]. Liver transaminases such as ASAT (aspartate transaminase) or SGOT (serum glutamic oxaloacetic transaminase), ALAT (alanine transaminase) or SGPT (serum glutamic pyruvic transaminase), serum alkaline phosphatase (SALP and Serum Total Proteins (STP) have still remained the gold standards for the assessment of liver injury[7]. So in the present investigation, there were significant ($p>0.0001$) increased levels of SGOT, SGPT, SALP, and Serum Total Proteins in paracetamol hepatic damage group in comparison to control group. The results clear indicate that these enzymes are cytoplasmic in location and are released into circulation after liver damage indicating hepatotoxicity. After treatment with 100 and 200 mg/kg Hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis* Rox for fourteen days, a significant decreased hepatic enzyme levels SGOT, SGPT, SALP, and Serum Total Proteins in *Ehretia laevis Roxb* treated group as compared with paracetamol treated group and come back near to control group.

The maximum hepatoprotective activity was exhibited ethyl acetate extract at 200mg/kg dose, which restored the levels of ALAT, ASAT and ALP very close to normal. The hepatoprotective effect of hydroalocoholic and ethyl acetate fraction of *Ehretia laevis* was also evaluated using histological studies. The histopathological analysis of liver tissue revealed fatty degeneration, lymphatic infiltration, and necrosis in control animals. The necrosis of the liver was significantly lower in the animals treated with Hydroalocoholic and ethyl acetate fraction of *Ehretia laevis* indicating the hepatoprotective effect of the above extracts. The liver of the animals treated with 200mg/kg of Hydroalocoholic extract and ethyl acetate fraction received significant hepatoprotection with near-normal appearance of the hepatic parenchyma including the portal areas.

CONCLUSION:

On the basis of above finding it is concluded that 100 and 200mg/kg hydroalocoholic (70%) extract and ethyl acetate fraction of *Ehretia laevis Roxb* is good efficacy and most effective drug for prevention of hepatic injury along with improved hematological biochemical parameters and decreased free radical generation and maintained antioxidants level during hepatic injury.

REFERENCES:

1. Hussain L, Ikram J, Rehman K, Muhammad T, Muhammad IB, Muhammad SHA. Turk J Biol. 2014; 38: 396-402.
2. Raj V, Mishra AK, Mishra A, Khan NA. Hepatoprotective effect of Prunus armeniaca L. (Apricot) leaf extracts on paracetamol induced liver damage in wistar rats. Pharmacognosy Journal. 2016; 8 (2): 154-158.
3. Salwe KJ, Mirunalini R, Mano J, Manimekalai K. Hepatoprotective and antioxidant activity of Murraya Koenigii leaves extract against paracetamol induced hepatotoxicity in Rats. Int J Basic Clin Pharmacol. 2017;6(6):1274-128.
4. Mowsumi FR, Rahman MM, Rahaman A, Hossain S. Preventive effect of ganoderma lucidum on paracetamol-induced acute hepatotoxicity in rats. Int J Sci Res. 2013; 5(3): 573-578.
5. Muhammad S, Fazal S. Comparative histopathology of acetaminophen induced hepatotoxicity in animal models of mice and rats. Pharmacology online. 2014; 3: 32-43.
6. Rathi C, Pipaliya N, Patel R, Ingle M, Phadke A, Sawant P. Drug induced liver injury at a tertiary hospital in India: etiology, clinical features and predictors of mortality. Ann. Hepatol. 2017; 16(3):442-450.
7. Dwivedi V, Mishra J, Shriv ASA Tava A. Efficacy study of livartho against paracetamol induced hepatotoxicity in adult sprague dawley rats. J Drug Metab Toxicol. 2015; 5(6): 1-7.
8. Sreejith GS, Ancheril IJ, Anitha MG, Ravishankar B. Hepatoprotective activity of Dushivishari agada in paracetamol induced hepatotoxicity of wistar rats. Int J Res. 2015; 6(5): 602-608.
9. Thakre R, Bhutada S, Chouragade B, Khobragde P, Harne K. Hydroalocoholic botanical properties of unexplored plant khandu chakka (ehretia laevis roxb.). Int. J. Ayur. Pharma Research. 2016; 4(7): 68-73
10. Rasika C. Torane, Gayatri S. Kamble, Asha A. Kale, Tushar V. Gadkari and Nirmala R. Deshpande. Quantification of dioctyl phthalate from ehretia laevis roxb by HPTLC. J Chem PharmRes.2011, 3(3):48-51
11. Jyothirmai N, Nagaraju B, Yaso DM, Sureshkumar JN, Swaruparani G. Evaluation of anti-Inflammatory and antibacterial activities of different solvent extracts of ehretia laevis roxb. J. Pharm. Sci. & Res. 2016; 8(8): 715-720.
12. Karluke SG, Khobragade P. Efficacy of folklore plant khanduchakka (ehretia laevis roxb) patra siddha tail in sandhivata. International Ayurvedic Medical Journal. 2017; 5(10): 3743-3751.
13. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014; 2 (5): 115-119.
14. Kamarudin NA, Markom M, Latip J. Effects of solvents and extraction methods on herbal plants phyllanthus niruri, orthosiphon stamineus and labisia pumila. Indian Journal of Science and Technology. 2016; 9 (21): 1-5.
15. Singh RP, Jain DA. Evaluation of antimicrobial activity of alcoholic and aqueous extracts of five plants used in traditional medicine in north India. Int. J. Pharm Tech Res. 2011; 3(1): 376-380.
16. Margret C, Jayakar B. Formulation and evaluation of herbal tablets containing ipomoea digitata linn. extract. International Journal of Pharmaceutical Sciences Review and Research. 2010; 3(1):101-120.
17. Byahatti VV, Khan AM, Patil SH, Choudhury A. Preliminary Phytochemical Investigation and In-vitro Antioxidant Activity of Bergenia ciliata Leaves. Research J. Pharm. and Tech. 3(2): April-June 2010; Page 399-401.
18. Uddandam Aruneswari, Sreerama Usha rani, M Aruna Devi, Galla Rajitha. Synthesis and Evaluation of Substituted Imidazolones for Antibacterial and Antioxidant Activities. Asian J. Research Chem. 4(2): February 2011; Page 257-259.
19. Beena P., Purnima S., Kokilavani R. In Vitro Anti Oxidant Study of Ethanolic Extract of Coldenia procumbens Linn. Asian J. Research Chem. 4(3): March 2011; Page 450-451.
20. Prithviraj Chakraborty, Suresh Kumar, Debarupa Dutta, Vikas Gupta. Role of Antioxidants in Common HeALATh Diseases. Research J. Pharm. and Tech.2 (2): April.-June. 2009.Page 238-244.
21. Rupali Kirtawade, Pallavi Salve, Anita Kulkarni, Pandurang Dhabale. Herbal antioxidant: Vitamin C. Research J. Pharm. and Tech. 3(1): Jan.-Mar. 2010; Page 58-61.
22. Sarika R. Deshmukh, Solomon Habtemariam, Prasad A. Wadegaonkar. Antioxidant and Antiproliferative Activity of Root Suspension Culture of Morinda citrifolia L. Research J. Pharm. and Tech.3 (4): Oct.-Dec.2010; Page 1189-1193.
23. Parul R, Kundu SK, Saha P. In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. The Pharma Innovation – Journal. 2013; 1(12): 83-88.
24. Dinai NJ, Naderi G, Soltanmohammadi N. The impact of extraction method on antioxidant activity of three herbs in four oxidative systems. J of Pharmacol & Clin Res. 2017; 4(1): 1-5.
25. Singh D, Mishra M, Gupta M, Singh P, Gupta A, Nema R. Nitric oxide radical scavenging assay of bioactive compounds present in methanol extract of centella asiatica. International Journal of Pharmacy and Pharmaceutical Science Research. 2012; 2(3): 42-44.
26. Palani Samy Hari Prasad, N. Ramakrishnan. Evaluation of Nitric Oxide Scavenging Activity of Rumex vesicarius L. Asian J. Research Chem. 4(9): Sept, 2011; Page 1482-1484.
27. Hardik Joshi, Manoj Pagare, Leena Patil, Vilasrao Kadam. In–Vitro Antioxidant Activity of Ethanolic Extract of Leaves of Buchanania Lanzan Spreng. Research J. Pharm. and Tech. 4(6): June 2011; Page 920-924.
28. Sim KS, Nurestri AM, Sinniah SK, Kim KH, Norhanom AW. Acute oral toxicity of pereskia bleo and pereskia grandiofolia in mice. Phcog Mag. 2010;6: 67-70.
29. Otimenyin SO, Olorunfemi PO, Audu Om, Sabo S. Sub-acute toxicity study of herbal blood tonic (hamebuild) in albino rats. Asian J Pharm Clin Res. 2013; 6(2): 78-83.
30. Singh A, Kumar GR, Gupta SS, Singh S, Rawat AS, Rao CV. Hepatoprotective potential of ziziphusoenoplia (l.) mill roots against paracetamol induced hepatotoxicity in rats. AJPCT. 2015; 3(01): 64-78.
31. Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam D, Sengupta P, Maiti BC, Maity TK. Evaluation of hepatoprotective and antioxidant activity of Ichnocarpus Frutescens (linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. Trop J Pharm Res. 2007; 6 (3): 755-765.
32. Pimple BP, Kadam PV, Badgujar NS, Bafna AR, Patil MJ. Protective effect of Tamarindus indica linn against paracetamol-induced hepatoxicity in rats. Indian J Pharm Sci. 2007; 69(6):827-83.
33. M Umadevi, C Maheswari, R Jothi, Sai Kishore Paleti, Y Srinivasa Reddy, R Venkata Narayanan. Hepatoprotective Activity of Flowers of Madhuca longifolia (Koen.) Macbr. Against Paracetamol-Induced Hepatotoxicity. Research J. Pharm. and Tech. 4(2): February 2011; Page 259-262.
34. Pradeep Kumar Samal, J.S. Dangi, Kedar Pd. Meena, N.R. Beck, Garima Maheshwari, Aswani Patel. Hepatoprotective Activity of Butea monosperma bark on Liver Damage Caused by Paracetamol in Rats. Research J. Pharm. and Tech. 4(5): May 2011; Page 771-774.
35. Mahesh KN, Wickramaratne MN, Wickramaratne DBM. Evaluation of antioxidant activity of five medicinal plants in Sri Lanka. Phcog J. 2014; 6(3): 49-54.