ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 03 | March - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A COMPARATIVE STUDY ON THE HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACTS OF IPOMOEA AQUATICA FORSK. AND CENTELLA ASIATICA LINN. IN ALBINO RATS

Pharma

Swagata Datta	Senior Resident, Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur, India, Pin code: 795004
Geetanjali Ningthoujam	Senior resident, Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur, India, Pin code: 795004
Christina Zosangpuii	Senior resident, Department of Pharmacology, Zoram Medical College, Falkawn, Aizawl, Mizoram, India, Pin code: 796005
Paonam Shyamasakhi Devi	Professor, Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur, India, Pin code: 795004
Leisangthem Tarinita Devi*	Associate Professor, Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur, India, Pin code: 795004 *Corresponding Author

ABSTRACT

Background: Drug induced liver injury is one of the important causes of acute liver failure. Acetaminophan, a commonly used over the counter drug, causes a potentially fatal, hepatic injury at overdose. The aim of the present study was to compare the hepatoprotective effect of ethanolic extracts of Ipomoea aquatica Forsk. (EEIA) and Centella asiatica Linn. (EECA) in albino rats by assessing liver function test and histopathological examination of liver tissue. **Methods:** Forty-two albino rats were divided into seven groups of six animals each. Group 1 (normal control) and 2 (hepatotoxic control) were administered 1% gum acacia orally, while group 3 (standard) was given silymarin orally at a dose of 100 mg/kg. Group 4 and 5 received EEIA orally at doses of 200 mg/kg and 400mg/kg respectively, while Group 6 and 7 received EECA orally at doses of 200 mg/kg and 400mg/kg respectively. All the groups received their respective drugs for 7days. Hepatotoxicity was induced in all groups except group 1 on day 7 by administering paracetamol orally at a dose of 2 g/kg. IBM SPSS version 21 was used for statistical analysis. P value <0.05 was considered statistically significant. **Results:** The findings suggest that both EEIA and EECA were able to exhibit hepatoprotective activity. It was also observed that EEIA at 400 mg/kg had slightly better hepatoprotective activity over EECA at 400 mg/kg. **Conclusion:** Further studies are needed to elucidate the exact mechanism of hepatoprotective effect offered by phytoconstituents and their role in prevention of liver injury.

KEYWORDS

Acetaminophan, Centella asiatica, Drug induced liver injury.

INTRODUCTION

Liver is the largest organ of the body, weighing 1–1.5 kg and representing 1.5–2.5% of the lean body mass.[1] Liver is very vulnerable to damage as it is the principal organ of biotransformation of many drugs. More than 900 drugs, toxins and herbs have been found to be responsible for liver injury directly or indirectly. Drug induced liver injury is the most common cause of withdrawal of a drug.[2,3]

Nonsteroidal anti-inflammatory drugs (NSAIDS) like paracetamol, aspirin, anti-tubercular drugs, anti-convulsants, oral contraceptives, anaesthetics, anti-cancers, anti-retrovirals, anti-fungals, statins and toxins like carbon tetrachloride etc are responsible for drug induced liver injury.[4] Liver injury is characterised by cellular necrosis, increase in lipid peroxidation, depletion in glutathione (GSH) level and increase in serum levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and lactate dehydrogenase (LDH).[1] Conventional hepatoprotective agents like N-acetyl cysteine, ursodeoxycholic acid etc are cautiously used due to their uncorroborated therapeutic effect and definite side effects. Indian traditional medicines are mainly based on the use of plant materials for treating various diseases.[5] *Ipomoea aquatica* Forsk. and *Centella asiatica* Linn. are two medicinal herbs with various phytoconstituents used by locals as medicine for the treatment of various disorders. *Ipomoea aquatica* Forsk. is used for diabetes, liver diseases, intestinal diseases, epistaxis, hypertension, helminthiasis, hyperlipidaemia, infectious diseases etc.[6] *Centella asiatica* Linn. is used for diarrhoea, liver diseases, fever, amenorrhoea, convulsion, diabetes, ulcer, anxiety, pain, various skin conditions such as leprosy, varicose ulcers, eczema, psoriasis.[7-9] This experimental study was done to compare the hepatoprotective effect of EEIA and EECA in paracetamol induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Study design

A non-randomised controlled experimental study was conducted in the Department of Pharmacology and Department of Pathology of RIMS, Imphal for a duration of two years after getting approval from the Institutional Animal Ethics Committee (IAEC), RIMS (Reg No. 1596/GO/a/12/CPCSEA). 42 adult wistar albino rats of either sex weighing 120-180 gm were included in the study while pregnant rats were excluded from the study.

Plant materials collection

The fresh leaves of *Ipomoea aquatica* Forsk. (Figure 1) was identified and authenticated by Professor and Head, Department of Life Sciences, Manipur University (Voucher no. 001351). The fresh leaves of *Centella asiatica* Linn. (Figure 2) was identified and authenticated by Botany Department, D.M College, Imphal (Acc. No. DMH 09.15).

Plant extract preparation

The plant materials were cleansed and dried under shade, powdered by mixer grinder. EEIA and EECA were be obtained by the extraction procedure using Soxhlet apparatus.[10] The percentage yield was 16% and 18% respectively.

Figure 1: *Ipomoea aquatica* Forsk. Figure 2: *Centella asiatica* Linn.

Toxicity studies

Acute toxicity testing was done in healthy albino rats according to Organisation for Economic Co-operation and Development (OECD) guidelines 423.[11] Limit test with 2000 mg/kg was carried out with six animals (three animals per step). There was no mortality observed at the dose of 2000 mg/kg till 14 days. So, maximum safe dose 200 mg/kg (1/10th of maximum test dose) and 400 mg/kg of both plants were selected for the study.

Phytochemical Screening

The preliminary phytochemicals analysis EEIA and EECA were carried out using standard procedure to identify various constituents.[1215]

Paracetamol induced hepatotoxicity

The hepatoprotective activity study was done by using paracetamol induced hepatotoxicity in albino rats.[16] 42 albino rats were obtained from Central Animal House, RIMS and divided into seven groups of six animals each. The rats were kept in polypropylene cages under conditions of controlled temperature (21-23°C) and 12 hour light: dark cycle for 1 week for acclimatization. They were fed with standard pellet diet with free access to water. The animals were fasted for 2 hour prior to drug administration. They were allotted to different groups with respective treatment as depicted in Table 1.

Table 1 Allotment of animals to different groups and their treatment

Groups	Drugs given
Group 1 (Normal Control)	1 % Gum acacia in distilled water p.o. for 7 days
Group 2 (Hepatotoxic Control)	1 % Gum acacia in distilled water (day 1-day 6) + Paracetamol 2 g/kg p.o. on 7th day
Group 3 (Standard)	Silymarin (100 mg/kg) p.o. for 7 days + Paracetamol 2 g/kg p.o. on 7th day
Group 4 (Test 1)	EEIA (200 mg/kg) p.o for 7 days + Paracetamol 2 g/kg p.o. on 7th day
Group 5 (Test 2)	EEIA (400 mg/kg) p.o. for 7 days + Paracetamol 2 g/kg p.o. on 7th day
Group 6 (Test 3)	EECA (200 mg/kg) p.o. for 7 days + Paracetamol 2 g/kg p.o. on 7th day
Group 7 (Test 4)	EECA (400 mg/kg) p.o. for 7 days + Paracetamol 2 g/kg p.o. on 7th day

All the drugs were suspended in 1% gum acacia in distilled water and administered orally in equal volumes of 0.5ml/100gm body wt. The animals were anaesthetised by light ether anaesthesia after 24 h of hepatotoxicity induction. About 2ml of blood were drawn from the retro-orbital sinus of all the albino rats by capillary tube and were allowed to coagulate for 30 minutes. The serum was separated by centrifugation at 3000 rpm for 20 min and analysed for SGOT, SGPT, ALP, total bilirubin and total protein by semi-auto analyser using commercial kits.[17,18] The livers were removed from the animals and a part of the tissues was fixed in 10% formalin for at least 24 hours. Then, the paraffin sections were prepared for histopathological examination using standard techniques. Tissues were cut into 5 µm thick sections by a rotary microtome. The sections were stained with haematoxylin-eosin dye (H&E), and studied for histopathological changes, i.e hepatocyte necrosis, cytoplasmic vacuolation, portal triad inflammation, sinusoidal dilatation and central vein dilatation.[19,20]

Statistical analysis

The data were entered in SPSS version 21.0 and results were analysed for statistical significance using ANOVA followed by Bonferroni test. P value less than 0.05 was considered significant.

RESULTS

Phytochemical screening

EEIA showed presence of tannins, flavonoids, starch, protein, amino acids, carbohydrates, saponins, steroids, alkaloids, triterpenoids and glycosides. EECA showed presence of tannins, flavonoids, amino acids, carbohydrates, saponins, steroids, alkaloids, triterpenoids and glycosides.

Hepatoprotective activity

The mean SGOT, SGPT, ALP, total protein and bilirubin of rats in the hepatotoxic group who received 1% gum acacia for 7 days followed by single dose of paracetamol (2g/kg) on the 7th day showed statistically significant rise ($p<0.001$) in SGOT, SGPT, ALP and bilirubin values with significant fall ($p<0.001$) in total protein as compared to the basal values in normal group.

Standard drug Silymarin reduced SGOT, SGPT, ALP and bilirubin values significantly ($p<0.001$) whereas total protein value was significantly increased ($p<0.001$) when compared to respective values of the hepatotoxic group.

The mean SGOT, SGPT, ALP, total protein and bilirubin levels in the test groups who received EEIA and EECA at doses of 200 mg/kg and 400 mg/kg showed gradual reduction in SGOT, SGPT, ALP and bilirubin values and increase in protein value which were statistically significant when compared to respective values of the hepatotoxic group. When EEIA 400 mg/kg and EECA 400 mg/kg were compared p-value was found to be statistically significant. EEIA at 400 mg/kg had slightly better hepatoprotective activity over EECA at 400 mg/kg.

The values of SGOT, SGPT, ALP, bilirubin and total protein of EEIA treated group (dose 400 mg/kg) were statistically insignificant when compared to respective values of silymarin treated group (Table 2).

Table 2: Effect of standard drug (Silymarin) and test drugs (EEIA and EECA) on liver enzymes on paracetamol induced hepatotoxicity

Groups	Biochemical tests expressed in Mean ± SEM				
	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	Total Protein(g/dl)	Bilirubin(mg/dl)
Normal control	39.29 ±2.09 *	31.11±2.55 *	103.16± 8.93 *	7.31±0.28 *	0.46±0.04 *
Hepatotoxic control	132.12±2.30	147.04±2.87	378.59± 15.27	3.46±0.27	1.19±0.11
Standard	57.29±1.16 *	66.76±1.81 *	165.57± 2.26 *	6.01±0.25 *	0.62±0.02 *
Test 1	94.28±3.19 *†	104.24±2.49 *†	257.05± 3.05 *†	4.66±0.09 **††	0.91±0.03 **††
Test 2	64.45±1.94 *‡	74.71±3.34 *‡	172.23± 2.98 *‡	5.84±0.04 *‡‡	0.72±0.01 *
Test 3	109.04±2.56 *†‡‡§	123.29±2.26 *†‡§	316.77± 14.12 **†‡‡§	4.74±0.26 **††§§§	0.96±0.02 ***††§§§
Test 4	78.97±2.76 **†‡‡§§‖	86.71±2.30 *†‡‡§§§‖	215.00± 5.42 *†† ‡‡‡§§§‖	4.86±0.04 **††‡‡‡	0.94±0.02 ***††‡‡‡

*p<0.001, **p<0.01, ***p<0.05 when other groups are compared to hepatotoxic control; †p<0.001, ††p<0.01 when standard is compared to test 1, 2, 3 and 4; ‡p<0.001, ‡‡p<0.01, ‡‡‡p<0.05 when test 1 is compared to test 2, 3 and 4; §p<0.001, §§p<0.01, §§§p<0.05 when test 2 is compared to test 3 and 4; ‖p<0.001 when test 3 is compared to test 4.

The histopathology of liver section of the rats from hepatotoxic group showed moderate hepatocyte necrosis, cytoplasmic vacuolation, portal triad inflammation, sinusoidal dilatation and central vein dilatation, ballooning degeneration. On the other hand, the liver section of standard group showed near normal hepatic parenchyma implying the protective effect of silymarin over paracetamol-induced hepatotoxicity. The test group treated with EEIA at dose 400mg/kg showed near normal hepatic architecture similar to standard group which further validates the hepatoprotective activity of EEIA at higher dose (Table 3). The photomicrograph of liver tissues (H &E stain) of different groups have been shown in Figure 3 (A-G).

Table 3: Histopathological scoring of different groups on paracetamol induced hepatotoxicity

Histopathological parameters	Normal control	Hepatotoxic control	Standard	Test 1	Test 2	Test 3	Test 4
Hepatocyte necrosis	-	++	-	+	-	+	-
Cytoplasmic vacuolation	-	++	-	+	-	+	+
Portal triad inflammation	-	++	+	++	+	++	+
Sinusoidal dilatation	-	++	-	+	-	++	+
Central vein dilatation	-	++	-	+	-	++	+

A: normal hepatic architecture – Group I; **B**: moderate hepatic necrosis, cytoplasmic vacuolation, portal inflammation, sinusoidal dilatation and central vein dilatation – Group II; **C**: normal hepatic architecture – Group III; **D**: mild hepatocyte necrosis, cytoplasmic vacuolation, sinusoidal dilatation, central vein dilatation and severe portal triad inflammation – Group IV; **E**: near normal hepatic architecture – Group V; **F**: mild hepatocyte necrosis and cytoplasmic

vacuolation, severe sinusoidal dilatation, central vein dilatation and portal triad inflammation – Group VI; **G**: mild cytoplasmic vacuolation, sinusoidal dilatation, central vein dilatation and portal triad inflammation – Group VII

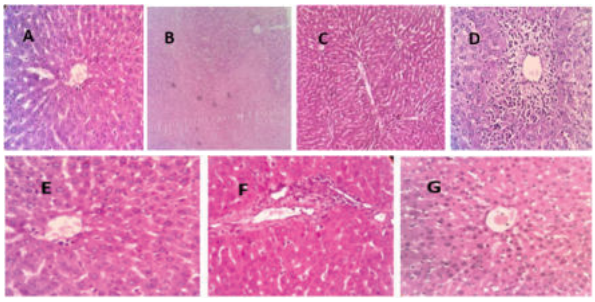

Figure 3: Photomicrograph of rat liver (H&E stain)

DISCUSSION

The mean SGOT, SGPT, ALP, total protein and bilirubin of rats in the hepatotoxic group who received 1% gum acacia for 7 days followed by single dose of paracetamol (2g/kg) on the 7th day were 132.12±2.30 IU/L, 147.04±2.87 IU/L, 378.59±15.27 IU/L, 3.46±0.27 g/dl and 1.19±0.11 mg/dl respectively. The results were similar to the studies conducted by Kumar SVS et al and Kiran PM et al.[21,22] The statistically significant rise ($p<0.001$) in SGOT, SGPT, ALP and bilirubin values with significant fall ($p<0.001$) in total protein as compared to the basal values in normal group was in accordance to the previous studies demonstrating the successful induction of hepatotoxicity by paracetamol in the present study. However, the variation in values with some studies may be explained by the difference in administered dose of hepatotoxin (paracetamol).

Silymarin is a standard drug used for hepatoprotective activity in hepatobiliary diseases and in hepatotoxicity due to drugs. It promotes protein synthesis, helps in regeneration of the liver tissue, controls inflammation, enhances glucuronidation and protects liver against glutathione depletion.[23] The SGOT, SGPT, ALP and bilirubin values of standard group were significantly reduced ($p<0.001$) whereas total protein value was significantly increased ($p<0.001$) when compared to respective values of the hepatotoxic group. The findings are similar to the studies conducted by Kiran PM et al and Lahon K et al.[22,24] This partial reversal of paracetamol induced hepatotoxicity by silymarin validates its use as a standard drug in the experiment.

The mean SGOT, SGPT, ALP, total protein and bilirubin levels in the test groups who received EEIA at doses of 200 mg/kg and 400 mg/kg showed gradual reduction in SGOT, SGPT, ALP and bilirubin values and increase in protein value which were statistically significant when compared to respective values of the hepatotoxic group. Silymarin treated group significantly reduced SGOT, SGPT, ALP and bilirubin and increased total protein when compared to the respective values of EEIA at dose 200 mg/kg. However, the changes in SGOT, SGPT, ALP, bilirubin and total protein after treatment with EEIA (dose 400 mg/kg) were statistically insignificant when compared to respective values of silymarin treated group. This suggests that hepatoprotective effect of EEIA at dose of 400 mg/kg is comparable to that of standard drug, silymarin.

The mean SGOT, SGPT, ALP, total protein and bilirubin levels in the test groups who received EECA at doses of 200 mg/kg and 400 mg/kg showed gradual reduction in SGOT, SGPT, ALP and bilirubin values and increase in protein value which were mostly statistically significant when compared to respective values of the hepatotoxic group. The reduction in SGOT, SGPT, ALP and bilirubin values and increase in total protein after treatment with silymarin were statistically significant when compared to respective values of EECA at both doses (200 mg/kg and 400 mg/kg).

When EEIA 400 mg/kg and EECA 400 mg/kg were compared p-value was found to be statistically significant. It was observed that EEIA at dose of 400 mg/kg was able to further reduce the SGOT, SGPT, ALP and bilirubin values as compared to EECA at dose of 400 mg/kg. The total protein of the group treated with EEIA at dose of 400 mg/kg was also higher than the group treated with EECA at dose of 400 mg/kg.

The histopathological findings obtained correlate with the biochemical findings. The findings of our study are in accordance to the studies done by Sivakumar V et al and Alkiyumi SS et al.[16,25]

The results of biochemical parameters and histopathological examination of both the plant extracts exhibited hepatoprotective effects but EEIA was found to be more effective than EECA. However, further studies are needed to elucidate the exact mechanism of hepatoprotective effect offered by the phytoconstituents and their clinical application in prevention or cure of liver injury or dysfunction.

REFERENCES

[1] Ghany MG, Hoofnagle JH. Approach to the patient with liver disease. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J, editors. Harrison's principles of internal medicine. 21st ed. New York: McGraw Hill Education; 2022. p. 2546-2552.
[2] Anstee QM, Jones DES. Drug and the liver. In: Ralston SH, Penman ID, Strachan MWJ, Hobson RP, editors. Davidson's principles and practice of medicine. 23rd ed. New York: Elsevier; 2018. p. 893-894.
[3] Pandit A, Sachdeva T, Bafna P. Drug induced hepatotoxicity: A review. J Appl Pharm Sci. 2012;2(5):233-243.
[4] David S, Hamilton JP. Drug induced liver injury. US Gastroenterology and Hepatology Review. 2010; (1):73-80.
[5] Gupta AK, Chitme H, Dass SK, Misra N. Antioxidant activity of *Chamomile recutita* Capitula methanolic extracts against CCl_4-induced liver injury in rats. J Pharmacol Toxicol. 2006;1(2):101-107.
[6] Malakar C, Nath Choudhury PP. Pharmacological potentiality and medical uses of *Ipomoea aquatica* Forsk.: A review. Asian J Pharm Clin Res. 2015;8(2):60-63.
[7] Saranya S, Nair AV, Prathapan MP, Neethu AS, Kumar NS. Phytochemical analysis of *Centella asiatica* L. leaf extracts. Int J Adv Res. 2017;5(6):1828-1832.
[8] Gohil KJ, Patel AJ, Gajjar AK. Pharmacological review on *Centella asiatica*: A potential herbal cure-all. Indian J Pharm Sci. 2010;72(5):546-556.
[9] Antony B, Santhakumari G, Merina B, Sheeba V, Mukkadan J. Hepatoprotective effect of *Centella asiatica* (L) in carbon tetrachloride-induced liver injury in rats. Indian J Pharm Sci. 2006;68(6):772-776.
[10] Knevel AM, Digangi FE. Jenkins' quantitative pharmaceutical chemistry. 7th ed. New York: McGraw Hill Book Co.; 1977.
[11] OECD. OECD guideline for testing of chemicals 423: Acute oral toxicity-acute toxic class [Internet]. 2001 [Cited 2022 July 20]. Available from http://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf.
[12] Kumar U, Kumar B, Bhandari A, Kumar Y. Phytochemical investigation and comparison of antimicrobial screening of clove and cardamom. Int J Pharm Sci Res. 2010;1(12):138-147.
[13] Shah B, Seth AK. Textbook of pharmacognosy and phytochemistry. 2nd ed. New Delhi: Elsevier; 2014.
[14] Dhanasekaran M, Abraham GC, Mohan S. Preliminary phytochemical and histochemical investigation on *Kigelia pinnata* DC. International Journal of Pharma Sciences and Research. 2014;5(7):413-419.
[15] Bargah RK. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma*. J Pharmacogn Phytochem. 2015;4(1):7-9.
[16] Sivakumar V, Sadiq AM, Bharathi SD. Hepatoprotective activity of *Centella asiatica* Linn. against paracetamol induced liver damage in experimental animals. Emer Life Sci Res. 2018;4(1):19-26.
[17] Parmar NS, Prakash S, editors. Screening methods in pharmacology. 2nd ed. New Delhi: Narosa Publishing House; 2006.
[18] Medhi B, Prakash A, editors. Practical manual of experimental and clinical pharmacology. 2nd ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2017.
[19] Galigher A, Kozloff E. Essentials of practical micro technique. Philadelphia: Lea & Febiger; 1971.
[20] Luna L. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 1st ed. New York: Blakiston Division McGraw-Hill; 1968.
[21] Kumar SVS, Sujatha C, Syamala J, Nagasudha B, Mishra SH. Protective effect of root extract of *Operculina turpethum* Linn. against paracetamol-induced hepatotoxicity in rats. Indian J Pharm Sci. 2006;68(1): 32-35.
[22] Kiran PM, Raju AV, Rao BG. Investigation of hepatoprotective activity of *Cyathea gigantea* (Wall. ex. Hook.) leaves against paracetamol-induced hepatotoxicity in rats. Asian Pac J Trop Biomed. 2012;2(5):352-356.
[23] Pradhan SC, Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. Indian J Med Res. 2006;124(5):491-504.
[24] Lahon K, Das S. Hepatoprotective activity of *Ocimum sanctum* alcoholic leaf extract against paracetamol induced liver damage in albino rats. Pharmacognosy Res. 2011;3(1):13-18.
[25] Alkiyumi SS, Abdullah MA, Alrashdi AS, Salama SM, Abdelwahab SI, Hadi AHA. *Ipomoea aquatica* extract shows protective action against thioacetamide-induced hepatotoxicity. Molecules. 2012;17(5):6146-6155.