Journal of Ayurveda and Integrative Medicine 12 (2021) 452–457

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original Research Article (Experimental)

Comparative hepatoprotective activity of detoxified roots of *Plumbago zeylanica* L. and *Plumbago rosea* L. in Wistar rats

A.R. Akhilraj [a, *], Sudhakar Bhat [b], B. Priyalatha [a], K.S. Vimala [a]

[a] Department of Dravyaguna (Ayurvedic Pharmacology), Amrita School of Ayurveda, Amritapuri, Amrita Vishwa Vidyapeetham, India
[b] Department of Pharmacology, Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Science, Udupi, India

ARTICLE INFO

Article history:
Received 21 October 2019
Received in revised form
23 November 2020
Accepted 3 April 2021
Available online 6 August 2021

Key words:
Citraka
Plumbago rosea L.
Plumbago zeylanica L.
Hepatoprotective activity
Paracetamol

ABSTRACT

Background: Paracetamol (acetaminophen) toxicity is considered to be one of the major causes of drug-induced hepatic failure. *Citraka* (*Plumbago rosea* L. and *Plumbago zeylanica* L.) was mentioned in Ayurveda classics as a remedy in liver disorders.
Objective(s): The aim of the study was to experimentally evaluate the comparative effect of hepatoprotective activity of detoxified root decoction of the two species of *Citraka* against paracetamol-induced hepatotoxicity in male Wistar albino rats.
Materials and methods: The hepatoprotective effect of *Citraka* decoction of two species was evaluated by the assessment of biochemical parameters such as SGOT, SGPT, alkaline phosphatase, total bilirubin, direct bilirubin, and serum creatinine. The study was also supported by histopathological assessment of liver sections.
Results: The results showed the elevated concentration of biochemical markers and histopathological degenerative changes in animals treated with paracetamol indicating severe hepatic damage; whereas, the treatment with decoction of both the species of *Citraka* showed significant reduction in the serum markers and regenerative changes in the histopathological specimens pointing towards its effectiveness as a hepatoprotective drug.
Conclusion: The present study showed *Citraka*'s effectiveness as a hepatoprotective drug and proved that the detoxified root decoction of *P. rosea* L. has a significant protective activity against paracetamol-induced hepatotoxicity than *P. zeylanica* L.

1. Introduction

The liver is a versatile organ which performs several diversified functions. It is the primary site of drug metabolism and an important target for drug-induced toxicity. The worldwide annual incidence of drug-induced hepatotoxicity is estimated to be 13.9–24.0 per 100,000 inhabitants [1]. Drug-induced toxicity should always be considered in the differential diagnosis of patients presenting with acute liver failure, jaundice or abnormal liver biochemistry. Among the drug-induced hepatic failure, paracetamol (acetaminophen) toxicity remains the most common cause. Silymarin is the standard proven hepatoprotective drug which is generally used in treating liver damage. However, recent studies have shown that the oral administration of silymarin at a dosage of 400 mg/kg causes cytotoxicity as well as changes in the biochemical parameters of blood [2]. Though conventional medicine has been successful in treating the condition, it brings some unwanted and undesired effects. Hence, search for herbal remedies with potent hepatoprotective activity and less side effects is the need of the hour. Treatment using Ayurvedic drugs becomes an alternative solution to provide effective treatment without any harmful effects. In *Pliha-Yakrt cikitsaadhyaya*, *Cakradatta* [3] has mentioned the use of *Citraka Kvatha* (decoction) in *Yakrt vikaras* (liver disorders). *Nighantu Ratnakara* [4] describes, *karma* of *Citraka* as *Yakrutrogahara*. Root of *Plumbago zeylanica* L. (*Sveta Citraka*) and *Plumbago rosea* L. (*Rakta Citraka*) of Plumbaginaceae family are used in Ayurveda as source plants of *Citraka*. Ayurveda, the science of life, emphasizes detoxifying techniques (*Sodhana*) for certain drugs to eliminate the toxicity while retaining its

* Corresponding author.
E-mail: akhilrajar999@gmail.com
Peer review under responsibility of Transdisciplinary University, Bangalore.

https://doi.org/10.1016/j.jaim.2021.04.002

potency [5]. Detoxifying methods of *Citraka* are mentioned in *Arogyakalpadrumam* [6] and Ayurvedic Pharmacopoeia of India (API) [7]. *Citraka* (*P. rosea* L. and *P. zeylanica* L.) is a very potent herbal drug and has well-proven hepatoprotective activity. However, there is a variation in the usage of the two species of *Citraka* across the states of India. API [8] and Quality Standards of Indian Medicinal Plants [9] specifies white-flowered *P. zeylanica* L. as the source plant of *Citraka*. This is contradictory to the use of red-flowered *P. rosea* L. as the source plant of *Citraka* in Kerala. Irrespective of the variety, it seems to be effective in producing the desired hepatoprotective activity. So far, no comparative study has been done on the hepatoprotective effects of above said two species of *Citraka*, but individual studies have been reported earlier [10–12]. Hence, the present study is significant in differentiating which species of *Citraka* possesses a better protection against paracetamol-induced hepatotoxicity.

2. Materials and methods

2.1. Plant materials

The roots of *Citraka* (2 species - *P. zeylanica* L. and *P. rosea* L.) were collected in June 2018 from the field at Pathanamthitta district, Kerala with the help of regional flora (Flora of Pathanamthitta [13]), and authenticated by a taxonomist. The roots of the plants were cut into smaller pieces and thoroughly washed using tap water (Figs. 1–2).

2.2. Detoxification of plant materials

Detoxification (*Sodhana*) of *Citrakamoola* i.e., root of two species of *Citraka* was carried out by one of the classical methods as specified in *Arogyakalpadrumam* and API. The cut pieces of 1 kg root each of both species *Citraka* were kept immersed in *Curnodaka* (lime water - CaCO3) for 24 h. Later the roots were taken out, washed with distilled water, dried and pre-served as '*Suddha Citraka*' - detoxified form of *Citraka*. After proper detoxification, the roots were subjected for shade-drying after which, they were powdered with a grinder and passed through mesh no. 60 to obtain coarse powder.

2.3. Preparation of medicine - decoction of Citraka [14]

To avoid any untoward effects, the *Kvatha* (decoction) was prepared on the same day. Forty-eight gm (1 *pala*) each of coarse powder of detoxified root of both *Citraka* species was taken, to which 16 times of water (768 ml) was added, then heated over *mandagni*, reduced to 1/8 quantity (96 ml) and filtered with a clean cloth.

2.4. Chemicals

Paracetamol (Dolo 650) was purchased from Micro Labs Ltd, Kumbalgodu Industrial Area, Bangalore. Silymarin (Silybon–70) was supplied by Micro Labs Ltd, Solan, Himachal Pradesh. All the reagents used in the experiment were of analytical grade and were bought from different firms.

2.5. Animals

Male albino rats (Wistar) with an average weight of 150–250 g were selected randomly for the study. They were obtained from animal house attached to S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka. They were maintained on feed of "Sai Durga feed and food, Bangalore" and tap water was given ad libitum. The animals were kept at controlled condition i.e., temperature (25 ± 2 °C) and humidity (50 ± 5%) and animals were exposed to natural day and night cycles. The experiments were carried out in conformity with the Institutional Animal Ethics Committee (IAEC) and after obtaining its permission (IAEC approval no: SDM¬CRA/IAEC/AM-DG-19).

2.6. Dose fixation

The dose of the drugs, vehicle, and toxicants were calculated by extrapolating the therapeutic dose of human to rat dose on the basis of surface area ratio by referring to the table of Paget and Barnes [15].

2.7. Grouping

The animals were divided into 5 groups of six rats each (n = 6).

Group I Vehicle treated: Animals received tap water.

Group II Paracetamol treated: Animals received paracetamol (3 g/kg, p.o.), 0.5% gum acacia and distilled water.

Group III Standard drug treated: Animals received Silymarin (50 mg/kg, p.o.) in addition to paracetamol.

Group IV Test drug 1: Animals received decoction of *P. rosea* L. (*Rakta Citraka*), TED (4.32 ml/kg, p.o.) in addition to paracetamol.

Group V Test drug 2: Animals received decoction of *P. zeylanica* L. (*Sveta Citraka*), TED (4.32 ml/kg, p.o.) in addition to paracetamol.

2.8. Treatment protocol

The test drugs (1 and 2) and standard drug were administered orally for 7 consecutive days and on the 7th day, one dose of the toxicant (paracetamol) was given orally to each group, except the water control group 1 h after test drug administration. The blood was taken after 48 h of toxicant paracetamol administration and sent for biochemical investigations. All the animals were sacrificed by cervical dislocation. Important organs like liver, heart, and kidney were dissected out, cleaned to remove extraneous tissues, blotted to remove blood stain and weighed. A piece of liver tissue was preserved in 10% formalin for histopathological processing.

2.9. Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM) of 6 animals per group. In the present study, parametric One-way Analysis of Variance (ANOVA) test was performed using GraphPad Prism 5.0. The minimum level of significance was identified at $p < 0.05$ with Dunnet's multiple 't' test as post hoc test.

3. Results

3.1. Gross observations in hepatoprotective study

All the animals were seen healthy and did not show any deviation from normal activities or behavior. The liver collected from Group I (normal control) was bright red in colour and Group II (paracetamol control) was pale red in colour and was enlarged in size. The liver collected from Group III (standard drug), IV (*P. rosea* L.) and V (*P. zeylanica* L.) were slightly enlarged and red in colour.

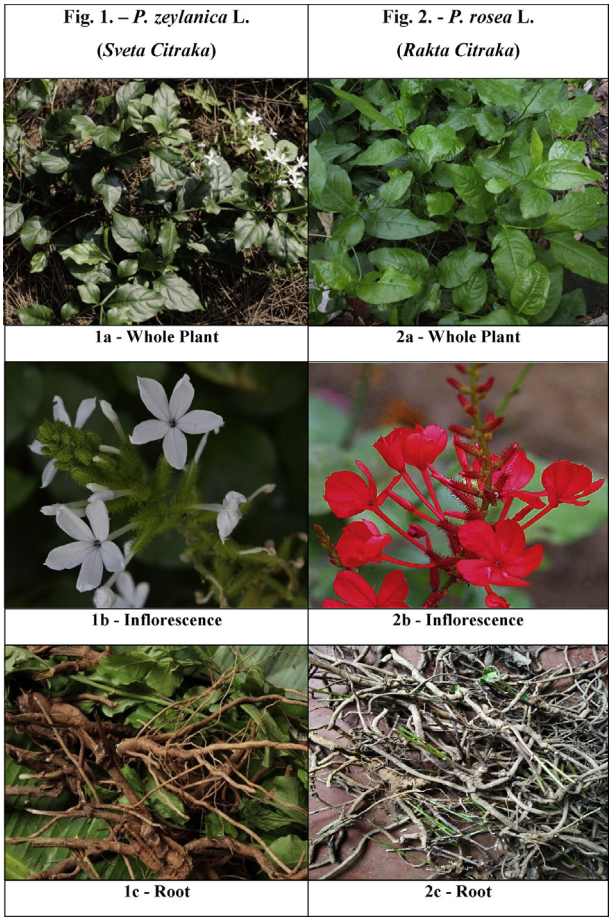

Fig 1 and 2. *P. zeylanica* L. and *P. rosea* L. comparison w.r.t (a) whole plant, (b) inflorescence, and (c) roots.

3.2. Serum biochemical parameters

The detailed effect of test drugs on serum enzymatic activity in paracetamol-induced liver damage in rats is elucidated clearly by Table 1.

3.3. Ponderal changes

The summarized results of ponderal changes are shown in Table 2.

3.4. Histopathology

Histopathological evaluation of livers of rats in normal control, paracetamol control, and treatment groups is depicted in Figs. 3–7.

4. Discussion

Even though *Citraka* (*P. rosea* L. and *P. zeylanica* L.) is not placed in the poisonous plant category, Ayurveda, the traditional system of medicine of India, advocated *sodhana* (detoxification) for this drug. *Citraka* contains plumbagin as the chief chemical constituent belonging to the class naphthoquinone, which is responsible for its corrosive effects. The 50% lethal dose (LD50) of plumbagin for the acute and sub-acute toxicity testing were 250 and 50–100 mg/kg body weight, respectively [16]. During detoxification process, using *Curnodaka* (lime water - CaCO3), excess of plumbagin diffuses into the *Curnodaka* [17].

Paracetamol (acetaminophen) is used globally for its analgesic and antipyretic properties; however, it causes acute liver damage if administered in overdose. The toxic effect of paracetamol on the liver is not only from paracetamol, but also from its metabolite N-acetyl P benzo quinoneimine (NAPQI) also known as N-acetyl limidoquimone. NAPQI depletes the liver's natural antioxidant glutathione and directly damages liver cells, leading to liver failure. Paracetamol is a direct hepatotoxin i.e., intoxication is dose-dependent and reproducible. Exposure of animals to higher doses produces centrilobular or massive hepatic necrosis followed by congestion and failure. The necrosis is also associated with damage to sub-cellular organelles. Thus, the drug is used as a typical hepatotoxin to produce hepatic injury experimentally [18,19]. The review of literature proved that the level of serum enzymes such as SGOT, SGPT and ALP gets elevated in paracetamol-induced hepatotoxicity.

In the present study also, similar elevation was observed. Increase in the levels of these marker enzymes in serum indicate damage to the liver cells. The standard drug Sylimarin, and the decoction of the two *Citraka* species viz., *P. rosea* L. and *P. zeylanica* L. significantly decreased the paracetamol-induced elevated levels of the enzymes in the treatment group, indicating regeneration of damaged liver cells or enhancement of structural integrity of hepatocytic cell membrane by the trial drugs. In the present study, a very significant elevation of SGOT level was observed in paracetamol-treated rats. It was found that both the test drug groups (1 and 2) and the standard group showed very significant decrease in the SGOT. The reversal percentage of test drug 1 (*P. rosea* L. - 49.84%) was even greater than that of Silymarin (44.22%), whereas test drug 2 (*P. zeylanica* L.) showed 42.20%. This

Table 1
Effect of test drugs on serum bio–chemical parameters.

SL. NO	BIOCHEMICAL PARAMETERS	PARACETAMOL CONTROL		STANDARD DRUG (Silymarin)		TEST DRUG 1 (*P. rosea* L.)		TEST DRUG 2 (*P. zeylanica* L.)	
		MEAN ± SEM	% Change	MEAN ± SEM	% Change	MEAN ± SEM	% Change	MEAN ± SEM	% Change
1	SGOT (IU/l)	237.27 ± 27.21**	91.58↑@	132.33 ± 7.93**	44.22↓#	119 ± 7.95**	49.84↓#	137.12 ± 17.30**	42.20↓#
2	SGPT (IU/l)	223.12 ± 22.68**	318.02↑@	94 ± 9.63**	57.87↓#	74.33 ± 4.41**	66.68↓#	64.87 ± 2.78**	70.92↓#
3	ALKALINE PHOSPHATASE (IU/l)	637 ± 50.71*	46.97↑@	476 ± 45.99	25.27↓#	514.83 ± 16.57	19.17↓#	450.75 ± 50.10	*20.23↓#
4	SERUM SUGAR (mg/dl)	95.75 ± 6.29	4.25↓@	144.37 ± 8.11**	50.78↑#	145.5 ± 6.29**	51.95↑#	137.62 ± 11.81**	43.72↑#
5	TOTAL PROTEIN(g/dl)	6.47 ± 0.53	2.99↑@	6.71 ± 0.07	3.66↑#	6.36 ± 0.18	1.74↓#	6.88 ± 0.21	6.36↑#
6	SERUM ALBUMIN(g/dl)	4.12 ± 0.07	2.17↑@	3.66 ± 0.04**	11.22↓#	3.71 ± 0.09**	10.01↓#	3.7 ± 0.09**	10.30↓#
7	SERUM GLOBULIN(IU/l)	2.35 ± 0.54	4.44↑@	3.05 ± 0.10	29.78↑#	2.65 ± 0.23	12.76↑#	3.18 ± 0.24	35.32↑#
8	TOTAL BILIRUBIN (mg/dl)	0.47 ± 0.02**	135↑@	0.32 ± 0.04*	31.91↓#	0.15 ± 0.01**	68.08↓#	0.28 ± 0.06**	40.42↓#
9	DIRECT BILIRUBIN (mg/dl)	0.25 ± 0.01**	257.14↑@	0.15 ± 0.02**	40.00↓#	0.06 ± 0.02**	76.00↓#	0.10 ± 0.03**	60.00↓#
10	SERUM UREA (mg/dl)	42.85 ± 2.45**	28.40↑@	39.33 ± 1.83**	8.21↓#	36.16 ± 1.32	15.61↓#	35 ± 1.71*	18.31↓#
11	SERUM CREATININE (mg/dl)	0.58 ± 0.02	14.64↑@	0.52 ± 0.02	10.56↓#	0.56 ± 0.01	4.26↓#	0.57 ± 0.02	2.04↓#
12	SERUM CHOLESTEROL (mg/dl)	81.62 ± 4.53	19.81↑@	80.25 ± 5.79	77.64↓#	78.25 ± 6.43	4.13↓#	87.75 ± 4.45	7.50↑#
13	SERUM TRIGLYCERIDES (mg/dl)	28.12 ± 5.78	31.83↓@	42.12 ± 13.03	49.77↑#	26.37 ± 5.16	6.22↓#	70.28 ± 9.25	1.49↑#

Data: MEAN ± SEM, **P < 0.01 *P < 0.05 ** - Very significant * - Significant @ - compared with normal control # - compared with paracetamol control.

Table 2
Effect of test drugs on ponderal changes.

SL. NO	PONDERAL INDEX	PARACETAMOL CONTROL		STANDARD (Silymarin)		TEST DRUG 1 (*P. rosea* L.)		TEST DRUG 2 (*P. zeylanica* L.)	
		MEAN ± SEM	% Change	MEAN ± SEM	% Change	MEAN ± SEM	% Change	MEAN ± SEM	% Change
1	BODY WEIGHT (gram)	−6.78 ± 1.26**	526.41↓@	2.39 ± 2.15**	135.25↑#	1.95 ± 0.17**	128.76↑#	1.64 ± 0.05**	124.18↑#
2	LIVER WEIGHT (gram)	9.54 ± 0.38*	22.30↑@	10.12 ± 0.64	6.07↑#	8.82 ± 0.50	7.54↓#	9.71 ± 0.23	1.78↑#
3	HEART WEIGHT (gram)	1.14 ± 0.04**	34.11↑@	1.05 ± 0.04	7.89↓#	1.01 ± 0.06	11.40↓#	1.06 ± 0.05	7.01↓#
4	KIDNEY WEIGHT (gram)	2.31 ± 0.11**	48.07↑@	2.20 ± 0.22	4.76↓#	2.01 ± 0.09	12.98↓#	2.02 ± 0.09	12.12↓#

Data: MEAN ± SEM, **P < 0.01 *P < 0.05 ** - Very significant * - Significant @ - compared with normal control # - compared with paracetamol control.

proves that the efficacy of test drug 1 (*P. rosea* L.) is more than test drug 2 (*P. zeylanica* L.) as an anti-hepatotoxic drug. Similarly, a very significant increase of SGPT level was observed in paracetamol control group. It was found that test drug 1, 2 and standard drug group showed very significant decrease in SGPT. Hence, both the test drugs are effective in reducing the elevated SGPT level also. The

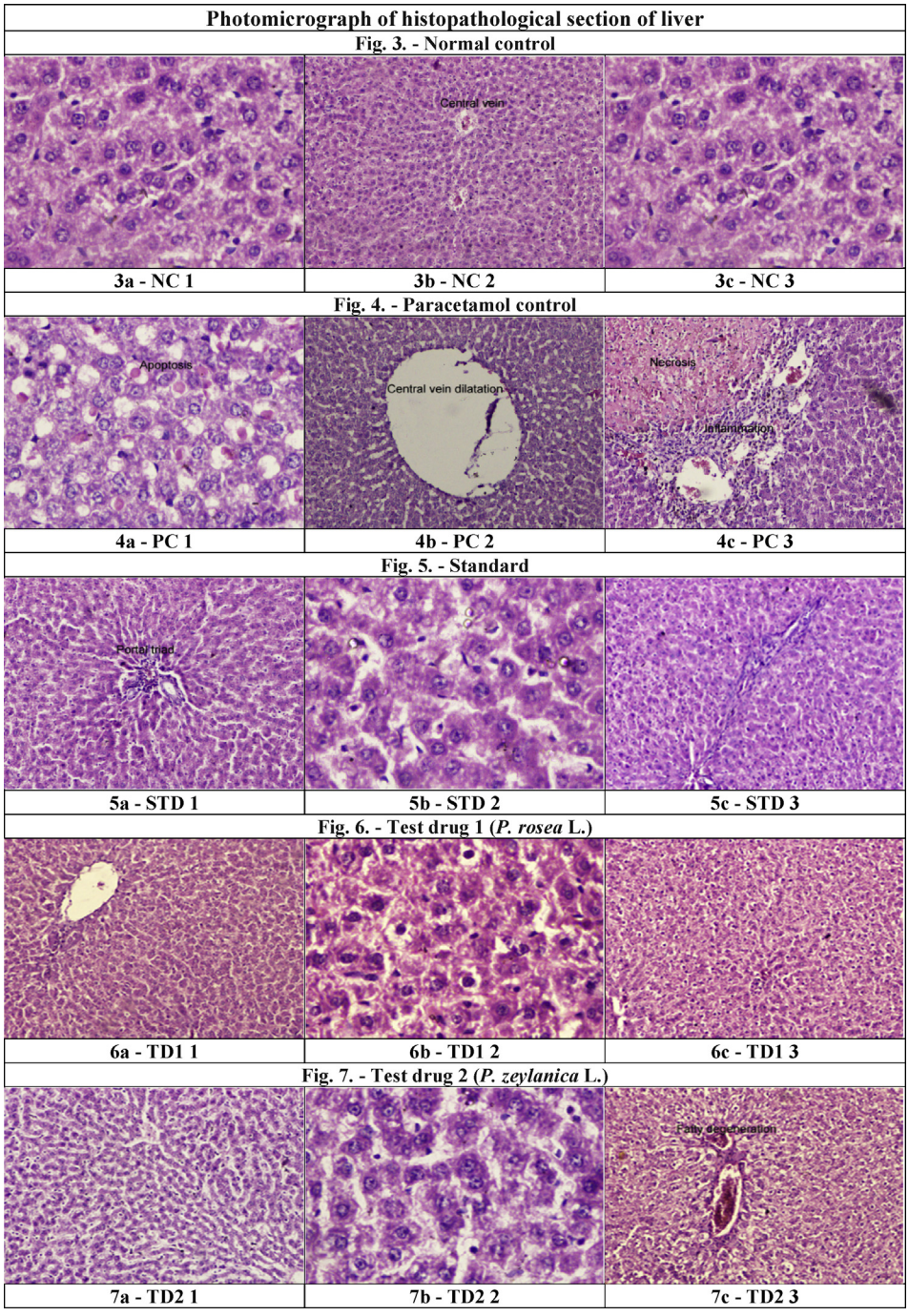

Fig 3–7. Photomicrograph of histopathological section of liver for normal control (3a–3c), paracetamol control (4a–4c), Standard (5a–5c), Test drug 1 (6a–6c), and Test drug 2 (7a–7c).

ALP level was found to be significantly increased in paracetamol control group in comparison to the normal control group. In Silymarin group, a non-significant decrease was observed compared to paracetamol control group. Test drug 1 showed a non-significant decrease in serum ALP level in comparison to paracetamol control group, whereas test drug 2 showed significant decrease. It might be due to the cytoprotective activity of the test drugs and showed its effect as an anti-hepatotoxic drug.

Decrease in the total and direct bilirubin after treatment with trial drugs indicated the effectiveness of decoction in the normal functional status of the liver. The total bilirubin was observed to be very significantly decreased in both test drug groups (1 and 2). The reversal percentage of test drug 1 (68.08%) was even greater than that of Silymarin (31.91%), whereas test drug 2 showed 40.02%. This proves that the efficacy of test drug 1 (*P. rosea* L.) is more than test drug 2 (*P. zeylanica* L.) as a hepatoprotective drug. In the case of direct bilirubin, the reversal percentage of test drug 1 (76%) was even greater than that of Silymarin (40%), whereas test drug 2 (*P. zeylanica* L.) showed 60%. This also proves that the efficacy of test drug 1 (*P. rosea* L.) is more than test drug 2 (*P. zeylanica* L.) as an anti-hepatotoxic drug.

The histopathological report of normal control group showed observations such as lobular arrangement of liver tissue. Each lobule consists of a central vein and portal triads along the periphery of lobules. Numerous sinusoids pass radially from the central vein and the spaces between the sinusoids contain liver cells which can be correlated with normal tissue architecture. Paracetamol control group revealed severe and extensive necrosis with fatty degenerative changes, inflammatory cell infiltration around the vessels, central vein dilatation, presence of cells exhibiting apoptopic changes, and hemorrhage streaks which can be connected with moderate to severe degeneration. Standard drug treated group showed mild inflammation around vessels. Some rat sections showed normal tissue architecture. One rat section showed severe patchy necrosis. Regenerative features like nuclear enlargement seen in some rat sections which can be correlated with mild changes to good protection in majority of the rats. Report of *P. rosea* L. treated group revealed mild inflammation in few rat sections. Few sections showed vein dilatation whereas few sections did not show any dilatation. Mild necrosis was seen in some rat sections. Regenerative changes like nuclear enlargement, nuclear hyper-chromatism were seen in some sections. Some rat sections showed normal tissue architecture. Fatty degeneration was seen in some sections which can be correlated with degenerations less severe than positive control and inflammation much reduced compared to positive control. *P. zeylanica* L. treated group revealed fatty degeneration and vein dilatation seen in some rat sections. Regenerative activity was seen in some sections. Sinusoidal dilatation was also seen in most of the sections which can be connected with degenerative changes, but to a lesser extent than positive control. Regenerative activity was also seen. Both the test drugs (*P. rosea* L. and *P. zeylanica* L.) were effective in histopathological level. On analyzing the report T1 (*P. rosea* L.) showed more therapeutic action than T2 (*P. zeylanica* L.).

4.1. Probable mode of action of drug

According to *Bhavaprakasha Samhita* [20] of *Bhavamishra*, though there is a difference in position of the organs, the aetiology and pathogenesis of *Yakrt* and *Pliha vikaras* are the same. In *Yakrt vikaras*, the *rakta dhatu* and *kapha dosa* become vitiated causing symptoms like loss of weight, low-grade fever, loss of appetite and reduced immunity. The predominant sign and symptoms are that of deranged *kapha* and *pitta*. Hence, the drug chosen for the treatment of *Yakrt vikaras* should effectively manage vitiated *kapha* and *pitta*.

Citraka pacifies *kapha dosa* because of *katu rasa* and *vipaka*; the *ushna virya* does *dipanapacana karma*, correcting *agni* and *pitta*. The *tikshna guna* initiates *srotosuddhi*. The *rasayana* property of *Citraka* rectifies *dhatu kshaya*. According to *Nighantu Ratnakara*, *Citraka* is said to be *Yakrutrogahara*. In this manner *Citraka* corrects the *nidana*, *samprapti* of *Yakrt vikaras*. Research studies proved that, the secondary metabolites present in *P. rosea* L. and *P. zeylanica* L. (alkaloids, triterpenes, sterols and zeylonone) enhances monooxygenase enzyme present in the liver, which enables the hepatoprotective activity.

5. Conclusion

The present study concedes that the test drugs (T1 - *P. rosea* L. and T2 - *P. zeylanica* L.) were having a comparable hepatoprotective activity with that of Silymarin. The trial drugs have helped in balancing the biochemical parameters studied almost as efficiently as the standard drug. On scrutinizing the biochemical parameters and histopathological data, it was concluded that the decoction from detoxified root of *P. rosea* L. has more therapeutic action than *P. zeylanica* L.

Source(s) of funding

None.

Conflict of interest

None.

Acknowledgement

Authors are greatly thankful to the Director, Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi, India for the ever-encouraging support and guidance during the experimental study.

References

[1] Suk KT, Kim DJ. Drug-induced liver injury: present and future. Clin Mol Hepatol 2012;18(3):249–57. PMID: 23091804.
[2] Banaee M, Sureda A, Mirvaghefi AR, Rafei GR. Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (*Oncorhynchusmykiss*). Fish Physiol Biochem 2011;37:885–96.
[3] Priya Vrat Sharma, Chakradatta Varanasi. Chaukhambha orientalia, edition. 2007. p. 325. 39th chapter.
[4] Krishna Bhishagavarya Navare Shastri, Ratnakara Nighantu. Collated with spacious notes by vasudeva laxman shastri Panshikar & Krishnaji vitthal soman. Bombay: Nirnaya Sagar Press, Edition year; 1936.
[5] Maurya SK, Seth A, Laloo D, Singh NK, Gautam DN, Singh AK. Śodhana: an Ayurvedic process for detoxification and modification of therapeutic activities of poisonous medicinal plants. Anc Sci Life 2015;34(4):188–97. https://doi.org/10.4103/0257-7941.160862. PMID: 26283803.
[6] Ramavarier Kaikulangara, Kalpadrumam Arogya. Thrissur. 8th ed. Sahithi Books; 2011. p. 382. 38th chapter.
[7] Ministry of Health and family welfare. Government of India, The Ayurvedic Pharmacopoeia of India. vol. I; 2001. p. 199–200. Part I.
[8] Ministry of Health and family welfare. Government of India, The Ayurvedic Pharmacopoeia of Indiavol. I. Citraka, Part I; 2001. p. 29.
[9] Gupta AK, Tandon N, Sharma M. Quality standards of Indian Medicinal Plants. In: Medicinal plants unit. Indian Council of Medical Research Pg; 2008. p. 208–26.
[10] Kanchana N, Sadiq MA. Hepatoprotective effect of Plumbago zeylanica on paracetamol induced liver toxicity in rats. Int J Pharm Pharmaceut Sci 2011;3(1). ISSN- 0975-1491.
[11] Tayubi IA, Desai A, Madar IH, Hussain AS. Hepatoprotective activity of Plumbago zeylanica linn. Against carbon tetrachloride induced hepatotoxicity in rats. Int J Scientific Innovat Aug, 2018;5(2):94–8.
[12] Eldhose B, Kose KJ, Mukale LS. Curative effect of Plumbago indica root extract on thioacetamide induced hepatotoxicity in experimental rats. Int J Phytomed 2017;9(1):138–43.
[13] Anil Kumar N, Sivadasan M, Ravi N. Flora of Pathanamthitta (Western Ghats, Kerala, India). Daya Publishing House; 2005. ISBN 81-7035-589-3.

[14] Srikantha Murthy KR. Sarngadhara Samhita by Sarngadhara. Varanasi: Chaukhambha Orientalia Reprint Edition; 2012. p. 56. Madhyama khanda [Chapter 2], Kvatha kalpana.
[15] Ghosh MN. Fundamental of experimental pharmacology. 3rd ed. Calcutta: scientific book agency; 2003. p. 192–7.
[16] Sumsakul W, Plengsuriyakarn T, Na-Bangchang K. Pharmacokinetics, toxicity, and cytochrome P450 modulatory activity of plumbagin. BMC Pharmacol Toxicol 2016;17:50. https://doi.org/10.1186/s40360-016-0094-5. PMCID: PMC5108082.
[17] Akhilraj AR, Suchitra Prabhu N, Priyalatha B, Vimala KS. Effect of *Śodhana* (An ayurvedic purification technique) on *Citraka (Plumbago zeylanica* Linn. and *Plumbago rosea* Linn.) with special reference to plumbagin content. J Pharmacogn Phytochem 2019;8(4):630–8. https://doi.org/10.22271/8-3-122.
[18] Parmar DV, Ahmed G, Khandkar MA, Katyara SS. Mitochondrial ATP phase: a target for paracetamol – induced hepatotoxicity. Eur J Pharmacol 1995;293:225–9.
[19] Tygtrup N, Jensen SA, Krorg B, Dalhoff K, Tygstrup N, Jensen SA. Expression of liver – specific functionsin rat hepatocytes following sublethal acetaminophen poisoning. J Hepatol (Amst) 1996;25:183–90.
[20] Srikantha Murthy KR. Bhavaprakasha Samhita of Bhavamisra. 3rd ed.vol. 2. Varanasi: Chaukhambha Krishnadas Academy; 2005. p. 565. Madhyama khanda [Chapter 33], Pliha Yakrt adhikara.