ISSN 2582 – 6050

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2020; 11(1):14-20

RESEARCH ARTICLE

Hepatoprotective Potential of *Tephrosia purpurea* in Thioacetamide-Induced Hepatotoxicity in Rats

Prashant Kumar Desai[1]*, Ritu Vishnoi Singhal[2], Peeush Singhal[3]

[1]*Department of Pharmacognosy, Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, Rajasthan, India,* [2]*Department of Botany, Chinmaya Degree College, BHEL, Ranipur, Haridwar, Uttarakhand, India,* [3]*Department of Pharmaceutical Sciences, Gurukula Kangri University, Haridwar, Uttarakhand, India*

Received: 25 October 2019; Revised: 20 November 2019; Accepted: 01 January 2020

ABSTRACT

Background: Hepatotoxicity ultimately leads to liver failure. Conventional treatment options for hepatotoxicity are limited and not safe. **Aim:** The present work has been designed to evaluate the hepatoprotective effects of ethanolic extract of the root of *Tephrosia purpurea* (Linn.) against thioacetamide-induced hepatotoxicity in experimental Wistar albino rats. **Materials and Methods:** The plant roots, *T. purpurea*, were collected from the local area of Jodhpur, Rajasthan, and verification was done by Botanical Survey of India (BSI), Jodhpur, Rajasthan, and a herbarium specimen was deposited in BSI with No. LMC/PM/PD-001. All other reagents and chemicals were of suitable analytical grade and were used as received. **Results:** On the basis of statistical analysis, both the doses (200 and 400 mg/kg b.wt) of the ethanolic extract of *T. purpurea* root shown significant hepatoprotective activity compare to negative control. The dose of 400 mg/kg b.wt showed better reduction in serum enzyme level compare to 200 mg/kg b.wt dose of the ethanolic extract of *T. purpurea* root. Results were determined by one-way analysis of variance (ANOVA non-parametric) followed by Dunnett's test with $P < 0.01$ considered statistically significant. **Conclusion:** Based on the results obtained, it may be concluded that the ethanolic extract of *T. purpurea* root has a significant protective effect on liver injuries.

Keywords: Bilirubin, hepatoprotective potential, *Tephrosia purpurea*, thioacetamide

INTRODUCTION

The liver is the dynamic organ of overriding significance intricate inside the protection of metabolic purposes and detoxing from exogenous and endogenous challenges, such as xenobiotic, drugs, viral infections, and continual alcoholism. If at some point of all such exposures to the above referred to challenges, the herbal defensive mechanism of the liver is overpowered, the end result is hepatic damage. Liver damage is always related to cell necrosis, increase in tissue lipid peroxidation, and depletion in the tissue glutathione tiers. In addition, serum levels of many biochemical markers such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), triglycerides, cholesterol, bilirubin, and alkaline phosphatase (ALP) are raised.[1] Significant advances in drug research notwithstanding effective pharmacological strategies are not available to counter deteriorating liver function that occurs as result of exposure to drugs and other environmental poisons. Indigenous systems of medicine have a strong repository of products that have been used traditionally to offer some sort of liver protection.[2]

Ayurveda, the ancient system of Indian medicine identified liver diseases quite early and recommended a number of herbal remedies. One such herb Sarapunkha (*Tephrosia purpurea*)

***Corresponding Author:**
Prashant Kumar Desai,
E-mail: prashant19481@gmail.com

is considered useful in bilious febrile attack, obstruction of liver and spleen.[3]

T. purpurea (Linn.) Pers. is widely growing small plant belonging to Fabaceae family. *T. purpurea* Linn. (Fabaceae) is a branched, sub-erect, perennial herb popularly known as Sarapunkha in Sanskrit, Purple Tephrosia in English, and Unali in Marathi.[4] The herb has white to purplish flowers and can be found in tropical regions. The plant showed central nervous system depressant, antibacterial, anti-allergic, and immunomodulator activity in rat and mice.[5-8] The root, leaves, seeds, and bark were used medicinally. *T. purpurea* is used as digestible, anthelmintic,[9] antipyretic,[10] astringent, and acrid and also used to cure diseases of liver,[11-13] spleen, heart, blood, ulcer, leprosy, and asthma.[14] The various parts of this plant were used as remedy for diabetes,[15] cancer,[16] stone problems,[17] and peptic ulcer,[18] in addition to its usefulness in the treatment of diseases related to oxidative stress and free radicals activity.[19]

The roots of plant were used in the treatment of various ailments such as dyspepsia, diabetes, rheumatism, asthma, diarrhea, urinary complaints, and cough.[20-27]

The available synthetic drugs to treat liver disorders in this condition may further worsen the liver damage as they too need to get metabolized in previously damaged liver. This increases the load on liver function and desired action of drug may not be observed. Steroids, vaccines, and antiviral drugs used as therapies for liver pathologies have potential adverse effects, especially if administered chronically or subchronically. Hence, developing pharmacologically effective agents from natural products has become a necessity by virtue of its comparatively low toxicity or fewer side effects. There are few plant-derived drugs in the market which are used in liver disorders that is why we have been designed the research to evaluate the hepatoprotective effects of the ethanolic extract of root of *T. purpurea* (Linn.) against thioacetamide-induced hepatotoxicity in experimental Wistar albino rats.

MATERIALS AND METHODS

This experiment was conducted in Pharmacognosy Laboratory, Department of Pharmacognosy, Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, Rajasthan, India - 342 003, from 2009 to 2014.

Materials

Anisaldehyde pure was obtained from Loba Chemie Ltd., Mumbai; gallic acid was from SD Fine Chem. Ltd., India, standard drug silymarin was obtained from Sigma-Aldrich Chemical Pvt. Ltd., Bengaluru. Thioacetamide and hepatotoxin were obtained from Sigma-Aldrich Chemical Pvt. Ltd., Bengaluru. Tween 80 was obtained from Nice Chemicals, Bengaluru. All other reagents and chemicals were of suitable analytical grade and were used as received.

Methods

Collection and preparation of the ethanolic extract of T. purpurea[28]

The plant roots, *T. purpurea*, were collected from the local area of Jodhpur, Rajasthan, and verification was done by Botanical Survey of India (BSI), Jodhpur, Rajasthan, and a herbarium specimen was deposited in BSI with No. LMC/PM/PD-001. The plant parts were cleaned, dried under shade, and powdered by a mechanical grinder. The powder was loaded into Soxhlet extractor (Tensil Glass Works, Bengaluru, India) in eight batches of 250 g each and was subjected to extraction for about 30–40 h with 95% ethanol. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure at a water bath temperature below 50°C to a syrupy consistency. Then, it was dried in the desiccator, finally yielding 78 g from 2.2 kg of dried plant roots. The shade-dried coarse powder of roots extracted in a Soxhlet extractor with 70% ethanol gave 20% extract.

Animals

Wistar albino rat (200–400 g) of either sex obtained from the animal house of Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, Rajasthan, and animals were kept in standard environmental conditions (temperature 23 ± 20°C, humidity 45 ± 5%) for 12 h dark-light

cycle and fed with standard diet of Altromin pellets and water *ad libitum*. The animals were housed individually in propylene cages containing sterile paddy husk (produced locally). The animal protocol was approved from the Institutional Animal Ethical Committee of Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, Rajasthan (Reg.No.-541/02/c/CPCSEA).

Hepatoprotective activity[29]

Adult Wistar rat (weighing 180–200 g, 8-week-old rat) of either sex was selected and kept in wire-bottomed cages at controlled temperature (21 ± 2°C) with 12 h light-dark cycle for at least a week before the treatment. The animals were given *ad libitum* access to food and water. Six rats were used in each group. Group I served as control (receive only vehicle). Group II served as negative control, thioacetamide (100 mg/kg b.wt, s.c) was administered on the 6th day of a total of 7-day study period to all the groups of animals except the control group (received vehicle only). Group III served as standard group and received silymarin (50 mg/kg, p.o). Groups IV and V served as two doses of the ethanolic extract of *T. purpurea* root (200 and 400 mg/kg b.wt).[11]

Biochemical analysis

The collected blood samples were used for the analysis of biochemical markers SGPT, SGOT, ALP, bilirubin (total and direct), cholesterol, and high-density lipoprotein (HDL) level. The different sections of liver stained with hematoxylin and eosin were observed microscopically for the evaluation of histopathological changes.

Statistical analysis

The results were expressed as mean ± standard error of the mean (n = 6). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test with $P < 0.01$ considered statistically significant.

RESULTS

The present study had been attempted to demonstrate the role of hepatoprotective activity of crude ethanolic extract of *T. purpurea* root in thioacetamide-induced hepatotoxicity in two different doses (200 and 400 mg/kg b.wt) of extract [Table 1].

Histopathology

Thioacetamide interferes with the movement of RNA from the nucleus to cytoplasm which may cause membrane injury. A metabolite of thioacetamide (s-oxide) is responsible for hepatic injury. Thioacetamide reduces the number of viable hepatocytes as well as the rate of oxygen consumption. It also decreases the volume of bile and its contents, i.e., bile salts, cholic acid, and deoxycholic acid.[27,28]

Table 1: Effects of the ethanolic extract of *Tephrosia purpurea* root on biochemical parameters in thioacetamide-induced hepatic injury in rats

Treatment	SGPT (u/l)	SGOT (u/I)	ALP (u/I)	Total bilirubin (g/dl)	Direct bilirubin (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)
Control (saline 0.5 ml p.o, 7 days)	39.70±1.92	74.66±2.23	107.79±1.84	0.59±0.01	0.28±0.01	62.37±1.9	32.83±1.2
Thioacetamide (TAA)	135.1±2.34	148.93±2.18	214.90±2.0	2.95±0.23	2.1±0.01*	97.67±2.6*	15.97±1.68
Silymarin (50 mg/kg p.o)+TAA	73.33±2.63*	99.74±1.81*	133.08±1.79*	1.85±0.04*	1.17±0.01*	67.49±1.77*	30.16±1.37*
TPEE (200 mg/kg b.wt, p.o)+TAA	111.8±2.31*	127.69±2.18*	196.04±2.53*	2.17±0.04*	1.3±0.02*	86.09±1.79*	18.87±1.61
TPEE (400 mg/kg b.wt, p.o)+TAA	103.8±4.15*	116.99±1.94*	168.89±1.69*	1.14±0.01*	0.51±0.02*	71.17±1.42*	21.98±1.64*

Results are expressed as mean±standard error of the mean, (n=6). Statistical significance was determined by one-way analysis of variance (ANOVA non-parametric) followed by Dunnett's test with P<0.01 considered statistically significant. Where *: Significant difference to thioacetamide. TAA: Thioacetamide, TPEE: *Tephrosia purpurea* ethanolic extract, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, HDL: High-density lipoprotein

Histological section of the control animals (Group I) showing normal hepatocytes with well-preserved cytoplasm, nucleus, and central vein. There were no sign of inflammation, fatty change, or necrosis in these animals [Figure 1]. Histological study of Group II animals showing hepatic injury induced by thioacetamide exhibits gross necrosis, ballooning of hepatocytes, and disturbed sinusoids [Figure 2].[29] Histological section of Group III (silymarin 50 mg/kg, p.o) animals showing almost normal liver lobule with no sign of necrosis in the centrizonal area observed [Figure 3]. Histological study of Group IV animals treated with *T. purpurea* 200 mg/kg showing less reduction of necrosed area and inflammatory infiltrates in the centrizonal area but fibrosis, necrosis, and ballooning of hepatocytes also observed [Figure 4]. Histological section of Group V animals treated with *T. purpurea* 400 mg/kg showing greater reduction of the necrosed area and sparse inflammatory cell infiltration around the central vein [Figure 5]. Ethanolic extract of *T. purpurea* at 400 mg/kg b.wt dose exhibits greater reduction of the necrosed area and sparse inflammatory cell infiltration around the central vein as compared to 200 mg/kg b.wt dose.[11] Effect of different doses of the ethanolic extract of *T. purpurea* (200 and 400 mg/kg b.wt) on various biochemical parameters (SGPT, SGOT, ALP, total bilirubin, direct bilirubin, cholesterol, and HDL level) compared with thioacetamide group is shown[30,31] in Table 1.

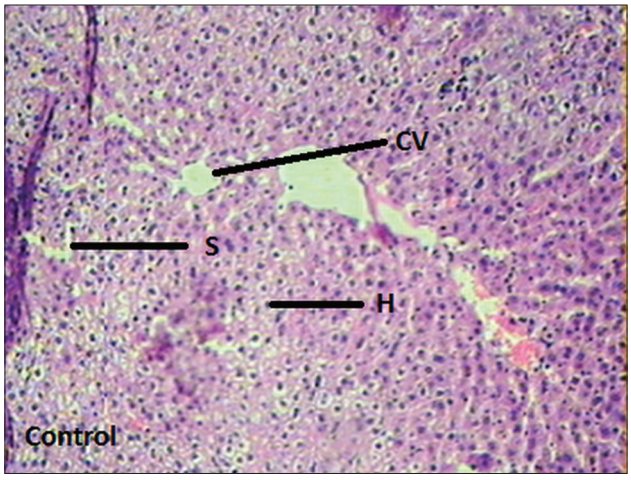

Figure 1: Histological section of Group I (control) animals. Where CV: Central vein, S: Sinusoids, H: Hepatocytes

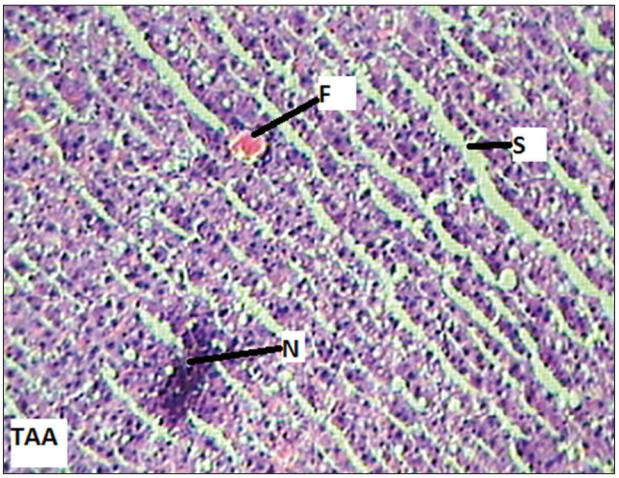

Figure 2: Histological section of Group II (negative control) animals. Where F: Fibrosis, S: Sinusoids, N: Necrosis

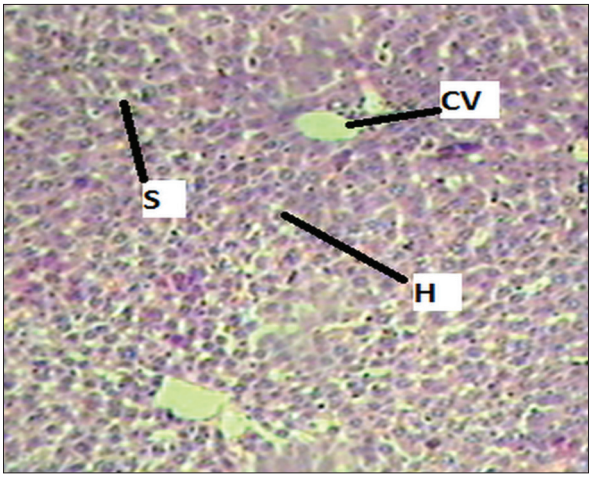

Figure 3: Histological section of Group III (silymarin 50 mg/kg) animals. Where CV: Central vein, S: Sinusoids, H: Hepatocytes

DISCUSSION

Human beings constantly struggle against the changing environmental condition to maintain optimum health and vigor throughout their life, during all the seasons. In addition, they are also exposed to various xenobiotics including drugs, chemical substances, and toxins and other stress.[21] Physiological homeostasis is preserved in spite of exposure to several such external challenges and endogenous aberrations.

Such challenges or aberrations cause various diseases. It is increasingly being realized now that a majority of the disease/disorders are mainly due

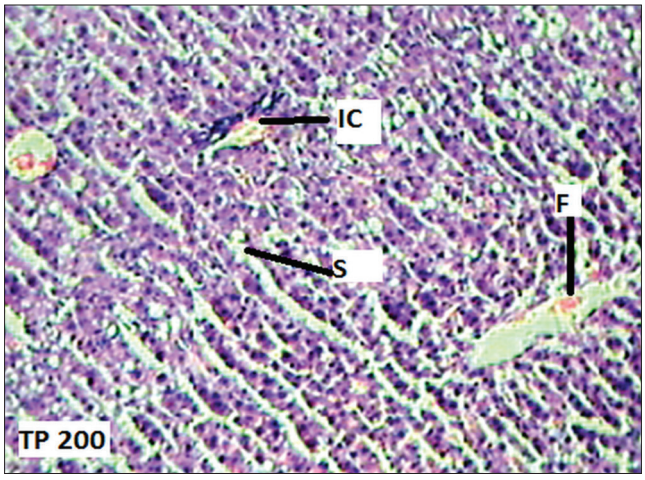

Figure 4: Histological section of Group IV (*Tephrosia purpurea* ethanolic extract 200 mg/kg) animals. Where *IC: Inflammatory cells in the centrizonal area, S: Sinusoids, F: Fibrosis

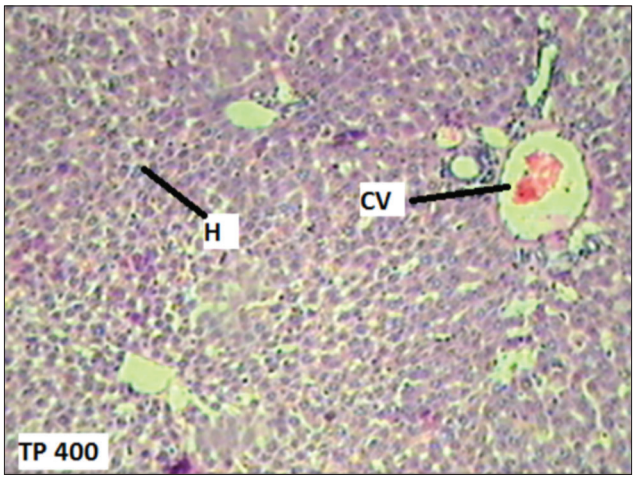

Figure 5: Histological section of Group V (*Tephrosia purpurea* ethanolic extract 400 mg/kg) animals. Where CV: Central vein, H: Hepatocytes

to imbalance between pro-oxidant (free radicals) and antioxidant homeostatic phenomenon. Liver is an organ that its physiological role and its self-protective mechanism are well developed. In spite of such balanced internal milieu, hepatic aberration, damage, and necrosis commonly occurring due to overexposure to hepatotoxic causes to such an extent that it overpowers the mechanism. Hepatotoxicity has been reported as one of the damages caused by free radicals. The present study had been attempted to demonstrate the role of hepatoprotective activity of crude ethanolic extract of *T. purpurea* in thioacetamide-induced hepatotoxicity at two different doses 200 and 400 mg/kg b.wt.

Measurement of the liver activity is considered as a good marker of hepatic function in animals and human in general. Assessment of liver damage can be made by evaluation of the plasmatic biochemical parameters such as SGPT, SGOT, ALP, bilirubin (total and direct), and cholesterol except HDL level. These substances leak into bloodstream during hepatopathy, which confirms the extent liver damage.[25] On the basis of biochemical parameters, the control group animals show normal level of all enzymes which indicate the normal cellular structure of liver [Table 2]. After treatment with thioacetamide, enzymes level was found to be elevated [Table 2] compared to normal group treated animals. Silymarin (standard group) treated animals group exhibited almost normal level of enzymes after thioacetamide-induced hepatotoxicity, compare to normal group treated animals. Ethanolic extract of *T. purpurea* at the dose of 400 mg/kg b.wt reduced the elevated level of enzymes, caused by thioacetamide-induced hepatotoxicity. On the basis of statistical analysis, ethanolic extract of *T. purpurea* at the dose of 400 mg/kg b.wt shown better reduction in serum enzyme level compare to 200 mg/kg b.wt dose of the ethanolic extract of *T. purpurea*. Results were determined by one-way ANOVA (ANOVA non-parametric) followed by Dunnett's test with $P < 0.01$ considered statistically significant against negative control, as shown in Table 1. Histological study of the control (received only vehicle) and standard (50 mg/kg p.o) group animals shown normal hepatocytes[11] with well-preserved cytoplasm, nucleus, and central vein [Figures 1 and 3]. Histological section of negative control animals in which hepatic injury induced by thioacetamide showed gross necrosis, ballooning of hepatocytes, and disturbed sinusoids [Figure 2]. *T. purpurea* (400 mg/kg b.wt.) showed greater reduction of the necrosed area and inflammatory cell infiltration around the central vein [Figure 5] as compared to 200 mg/kg b.wt dose [Figure 4]. Same procedure was adopted for the determination of hepatoprotective activity of the ethanolic extract of *T. purpurea* root against thioacetamide-induced hepatotoxicity animal model.[31] The results revealed that alcoholic extract of *T. purpurea* root (100 and 200 mg/kg b.wt) has significantly decrease the

Table 2: Design of the thioacetamide-induced hepatotoxicity animal model study

Groups	Treatments
I	Control (receive vehicle only)
II	Thioacetamide (100 mg/kg b.wt, s.c)
III	Silymarin (50 mg/kg b.wt)
IV	Ethanolic extract of *Tephrosia purpurea* root (100 mg/kg)
V	Ethanolic extract of *Tephrosia purpurea* root (200 mg/kg)

SGPT, SGOT, ALT, total bilirubin, direct bilirubin, cholesterol level, and increase the serum level of HDL. On the basis of biochemical parameters, the control group animals show that normal level of all enzymes indicates the normal cellular structure of liver. After treatment with thioacetamide, enzymes level was found to be elevated except HDL (18.15 ± 1.71 mg/dl) compared to normal group treated animals. Silymarin (standard group) treated animals shown almost normal level of enzymes after the induction of liver toxicity induced by thioacetamide, compare to normal group treated animals. Ethanolic extract of *T. purpurea* at the dose of 200 mg/kg b.wt reduced the elevated level of enzymes [Table1] except HDL caused by thioacetamide-induced hepatotoxicity. On the basis of statistical analysis, both the doses (100 and 200 mg/kg b.wt.) of the ethanolic root extract of *T. purpurea* shown significant hepatoprotective activity compare to negative control. Ethanolic extract of *T. purpurea* root at the dose of 200 mg/kg b.wt was shown better reduction in serum enzymes level compare to 100 mg/kg b.wt dose. Results were determined by ANOVA (non-parametric) followed by Dunnett's test with $P < 0.01$ considered statistically significant. Effect of *T. purpurea* root ethanolic extract on biochemical parameters in thioacetamide-induced hepatic injury in rats is shown in Table 1.

CONCLUSION

Based on the results obtained, it may be concluded that the ethanolic extract of *T. purpurea* root has a significant protective effect on liver injuries. Literature review also revealed that the root of *T. purpurea* contains flavonoids. The ethanolic extract of *T. purpurea* root has also shown the positive test of flavonoids. Therefore, there is a possibility that the root extract may possess antioxidant property, which may be involved in the hepatoprotective property.

SIGNIFICANT STATEMENT

Indigenous systems of medicine have a strong repository of plants that have been used traditionally to offer some sort of liver protection. Less side effects, easy availability, and highly economic factors make the herbal drug better alternative of synthetic drugs. It is considered worthwhile to investigate some indigenous plants which have reputation in Ayurveda and folk medicines. On the basis of the literature survey, it was found that *T. purpurea* and *Plumbago zeylanica* root play an important role to treat liver disease, but still no scientific attempt has been made to investigate the hepatoprotective activity of the plants. Hence, the present work is an attempt to study the hepatoprotective activity of *T. purpurea* root extract. Based on the results obtained, it may be concluded that the ethanolic extract of *T. purpurea* root has a significant protective effect on liver injuries. Literature review also revealed that the root of *T. purpurea* contains flavonoids. The ethanolic extract of *T. purpurea* root has also shown the positive test of flavonoids. Therefore, there is a possibility that the root extract may possess antioxidant property, which may be involved in the hepatoprotective property.

ACKNOWLEDGMENT

We thankful to Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, Rajasthan, India, for providing experimental facilities.

AUTHORS' CONTRIBUTIONS

PD was involved in design of research protocol, statistical assessment of all the results, and drafted the manuscript. PS assisted in design, analysis, and interpretation of the data and coordinated the experiments, and drafting the manuscript. RVS was involved in collection of plant and preparation of the ethanolic extract of *T. purpurea*.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

REFERENCES

1. Setty SR. Hepatoprotective activity of *Calotropis procera* flower. Fitoterapia 2007;34:134-61.
2. Mohammad A. Current status of hepatoprotective medicinal plants: Natural products used in hepatoprotective remedies in traditional medicine. Int J Curr Trends Drug Dev Ind Pharm 2018;2:11-41.
3. Shah R, Parmar S, Bhatt P. Evaluation of hepato-protective activity of ethyl acetate fraction of *Tephrosia purpurea*. Pharmacol Online 2011;3:188-94.
4. Burke MD. How to interpret liver function tests. South Sudan Med J 2017;10:40-3.
5. Vikas V, Bhangale SC, Vijay RP. Studies on immunomodulatory activity of *Ficus Carica*. Int J Pharm Pharm Sci 2010;2:97-9.
6. Neeraj T, Madhu S, Saraswathy A, Shakila R. Quality Standards of Indian Medicinal Plants Vol. 8. New Delhi: Medicinal Plants Unit ICMR; 2010.
7. Cohen SM, Ahn J. Review article: The diagnosis and management of alcoholic hepatitis. Aliment Pharmacol Ther 2009;30:3-13.
8. Shenoy S, Shwetha K. Evaluation of antiinflammatory activity of *Tephrosia purpurea* in rats. Asian Pac J Trop Med 2010;3:193-5.
9. Chinniah A, Mohapatra S, Goswami S, Mahapatra A, Kar SK, Mallavadhani UV, *et al*. On the potential of *Tephrosia purpurea* as anti-*Helicobacter pylori* agent. J Ethnopharmacol 2009;124:642-5.
10. Hugar S, Nanjappaiah HM, Kalyane N, Chowdhary M, Pandarinath. Phytopharmacology of *Tephrosia purpurea* Linn: An overview. Pharmacologyonline 2011;3:1112-40.
11. Khatri A, Garg A, Agrawal SS. Evaluation of hepatoprotective activity of aerial parts of *Tephrosia purpurea* L. and stem bark of *Tecomella undulata*. J Ethnopharmacol 2009;122:1-5.
12. Verma N, Neeraj, Singh J. Evaluation of hepatoprotective activity of *Tephrosia purpurea* Linn. Stem. Int Educ Appl Sci Res J 2017;2:5-6.
13. Gunjegaonkar SM, Saraswathi CD, Hrishikeshavan HJ, Harish MS, Nargund LV. Hepatoprotective and antioxidant activity of *Tephrosia purpurea* whole plant aqueous extract. Pharmacologyonline 2010;2:568-74.
14. Mukherjee PK, Rai S, Bhattacharya S, Bebnath PK, Biswas TK, Jana U. Clinical study of Triphala-awell-known phytomedicine from India. Iran J Pharmacol Ther 2007;5:51-4.
15. Pavana PB, Sethupathy SA, Santha KA, Manoharan SB. Complementary and alternative medicines. Afr J Tradit 2009;6:78-86.
16. Baranwal A, Mazumder A, Chakraborthy GS, Gupta S. Phytopharmacological uses of *Tephrosia purpurea*-a review. Pharmacophore 2014;5:658-65.
17. Bhadada SV, Goyal RK. Effect of aqueous extract of *Tephrosia purpurea* on cardiovascular complications and cataract associated with streptozotocin-induced diabetes in rats. Indian J Pharm Sci 2015;77:522-9.
18. Sumbul S, Ahmad MA, Mohd A, Mohd A. Role of phenolic compounds in peptic ulcer: An overview. J Pharm Bioallied Sci 2011;3:361-7.
19. Pavan P, Sethupathy S. Protective role of *Tephrosia purpurea* ethanolic seed extract on glycoprotein component in STZ induced diabetic rat. Int J Pharmacol 2008;4:114-9.
20. Gopalakrishnan S, Vadivel E, Dhanalakshmi K. Pharmacognostical standardization of *Tephrosia purpurea* root. J Herb Med Toxicol 2009;3:73-8.
21. Rakesh V, Navreet G. Drugs and the liver. Anaesthesia Intensive Care Med 2018;19:30-4.
22. Lin S, Smith BS. Drug dosing considerations for the critically ill patient with liver disease. Crit Care Nurs Clin North Am 2010;22:335-40.
23. D'Argenio G, Cariello R, Tuccillo C, Mazzone G, Federico A, Funaro A, *et al*. Symbiotic formulation in experimentally induced liver fibrosis in rats: Intestinal microbiota as a key point to treat liver damage? Liver Int 2013;33:687-97.
24. Tapasya S, Kunzang C. Clinical Biochemistry Clinical Enzymology and its Applications. New Delhi: Department of Biochemistry, All India Institute of Medical Sciences; 2007.
25. Akash M, Kannappan N, Santhiagu A. Evaluation of hepatoprotective activity of methanolic extract of *Justicia beddomei* (Clarke) Bennett against in and rifampicin induced hepatotoxicity. Am J Pharm Tech Res 2014;1:870-8.
26. Sreejith M, Akash M, Kannappan N, Harikrishnan V. Hepatoprotective activity of methanolic extract of *Flacourtia sepiaria* ROXB against D-galactosamine induced oxidative stress in rats. Int J Pharm Res Health Sci 2018;6:2143-47.
27. Kemelo MK, Wojnarová L, Kutinová Canová N, Farghali H. D-galactosamine/lipopolysaccharide-induced hepatotoxicity downregulates sirtuin 1 in rat liver: Role of sirtuin 1 modulation in hepatoprotection. Physiol Res 2014;63:615-23.
28. Bhavsar SK, Joshi P, Shah MB, Santani DD. Investigation in to hepatoprotective activity of *Citrus limon*. Pharm Biol 2007;45:303-11.
29. Das K, Kathiriya AK. Hepatoprotective activity of *Stevia rebaudiana* Bert. Leaves against thioacetamide induced toxicity. Turk J Pharm Sci 2012;9:343-52.
30. Janey A, Muhammad M, Badruddeen, Yasmeen J, Bagga P, Muhammad A. Hepatoprotective potential of ethanolic extract of *Aquilaria agallocha* leaves against paracetamol induced hepatotoxicity in SD rats. J Tradit Complement Med 2017;7:9-13.
31. Mishra G, Khosa RL, Singh P, Jha KK. Hepatoprotective potential of ethanolic extract of *Pandanus odoratissimus* root against paracetamol-induced hepatotoxicity in rats. J Pharm Bioallied Sci 2015;7:45-8.