Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

EVALUATION OF PHYTOCHEMICAL, ANTI-OXIDANT AND HEPATOPROTECTIVE ACTIVITIES OF METHANOLIC EXTRACT OF *PAVETTA INDICA*

H. Seena*[1], N. Kannappan[1], P. Manojkumar[2]

[1*]Department of Pharmacy, Annamalai University, Annamalai Nagar 608002, Tamil Nadu, India.
[2]Department of Chemistry, The Dale View College of Pharmacy and Research Centre, Trivandrum, Kerala, India.

ABSTRACT

The aim of the work was to investigate the phytoconstituents, antioxidant property and hepatoprotective activity of *Pavetta indica* herb. The aerial parts were dried, powdered and extracted by using a soxhlet extractor. Extracts were screened for phytoconstituents and total antioxidant property was evaluated by phosphomolybdenum method. *In vitro* hepatoprotective activity was determined by MTT assay method and *in vivo* study was performed by CCl_4 induced hepatotoxicity in male wistar albino rats. The results reveal that the methanolic extract have a potential antioxidant and hepatoprotective activity.

KEYWORDS

Pavetta indica methanolic extract, Antioxidant, Hepatoprotective, SGOT, SGPT and Chang liver cell line.

Author for Correspondence:

Seena H,

Department of Pharmacy,

Annamalai University,

Annamalai Nagar, Tamil Nadu, India.

Email: seenakannan02@gmail.com

INTRODUCTON

In our body, liver is one of the most important organs. It plays a primary role in metabolism, secretion, storage, and detoxification of endogenous and exogenous substances. Liver disease can occur through several mechanisms. Infection by hepatitis B virus or hepatitis C virus, alcohol abuse, and obesity are some of the major causes of liver disease[1]. In 1936, it was reported for the first time that carbon tetrachloride produced liver injury in rats[2]. Since then carbon tetrachloride is being used to induce hepatotoxicity as an experimental model for acute and chronic liver failure. CCl_4 is

metabolized by CYP2E1, CYP2B and CYP3A to form trichloromethyl radical (CCl_3). This radical (CCl_3) also binds to cellular molecules damaging crucial cellular progressions and also react with oxygen to form trichloromethylperoxy radical CCl_3OO, a highly reactive species. CCl_4 metabolite cause hepatic injury and acute liver injury model[3].

Antioxidants are frequently used to prevent oxidative damage caused by free radicals and thus lower the risk of liver diseases[4]. Use of antioxidants helps in preventing or retarding the appearance of liver diseases. Flavanoid, a class of hydroxylated phenolic substances, reported as antioxidants in plants, has the ability to scavenge free radicals and reduce its formation. The flavanoids exhibit antioxidant activity due to their ability to transfer electron free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidases[5].

Even though modern medicine is more advanced, there are very less restorative drugs for hepatic function that provide complete protection of the organ or regenerate hepatic cells. So it is essential to spot substitutes for the treatment of liver diseases, which are more effective and less toxic. Worldwide plants and natural products have been used traditionally for the prevention and treatment of liver diseases. Natural products have received appreciable recognition in recent years due to their multiple pharmacological properties[6]. The plant *Pavetta indica* is traditionally used as diuretic, purgative, antiulcer, hepatoprotective, prescribed to treat constipation, jaundice, headache, urinary diseases, dropsy, dysentery and toothache. The scientific evaluation of plants is necessary for modern medicine to emerge with productive pharmaceuticals for the treatment of liver diseases. Thus the current study was aimed to scientifically evaluate the antioxidant and hepatoprotective activity in *Pavetta indica*.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

The aerial parts of the plant was collected from Tirunelveli district, Tamil Nadu, and were dried in shade for 3 weeks, sorted out, powdered using a mechanical grinder and passed through a 40 mesh sieve. The above materials were stored in an airtight container, and used for further studies.

PLANT AUTHENTIFICATION

The plant was identified and authenticated by Mr. Chelladurai, Research officer- Botany, Central Council for Research in Ayurveda and Siddha, Government of India.

EXTRACT PREPARATION

Using soxhlet extraction method, the powdered drug was processed with solvents of increasing polarity. The extracts of petroleum ether, chloroform, ethyl acetate and methanol were subjected to preliminary phytochemical study.

PRELIMINARY PHYTOCHEMICAL STUDY

The different extracts of aerial parts of *Pavetta indica* was subjected to preliminary phytochemical screening to find the presence of phytoconstituents like alkaloids, carbohydrates, flavanoids, phenolic content, terpenoids and saponins[7,8].

ANTIOXIDANT ACTIVITY

Total Anti-oxidant activity was studied by Phosphomolybdenum Method[9]. In the reaction tubes 1mg/ml of extract was mixed with 3 ml reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate).The reaction solution were incubated at 95°C for 90 min. The absorbance of the resulting solution was measured at 695 nm using a ultra violet-visible spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) was used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid with methanol.

HEPATOPROTECTIVE ACTIVITY

IN VITRO METHOD

Chang liver cell line was procured from National Centre for Cell Sciences, Pune and maintained in Dulbecos modified Eagles medium (Hi media). The cell line was cultured in 25 cm^2 tissue culture flask

with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator. After gaining sufficient growth, CCl_4 (0.1%) was incorporated to induce toxicity and incubated for one hour. The prepared extracts were five times serially diluted by two fold dilution that is 100µg, 80µg, 40µg, 20µg, and 10µg in 100µl of 5% DMEM and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

HEPATOTOXICITY BY MTT ASSAY METHOD

After 24 hours of incubation , the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was slightly shaken, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After 4 hours of incubation, the supernatant was removed and 100µl of MTT solubilization solution DMSO was added and the wells were mixed by pipetting up and down in order to solubilize the formazan crystals. The absorbance was measured by using microplate reader at a wavelength of 570 nm. The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{OD sample}}{\text{OD Control}} \times 100$$

***IN VIVO* METHOD**

EXPERIMENTAL ANIMALS

Healthy male Wistar rats weighing 200g were obtained from the Animal house of Govt. Veterinary College, Mannuthy Polypropylene cages were used for housing the animals by providing standard environmental conditions recommended in CPCSEA guidelines. The animals were fed with normal laboratory chow standard pellet diet and had free access to water. The animals were habituated for 1 week before starting the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (Approval No. 1118/PO/Re/S/07/CPCSEA dated 14/02/2017), The Dale View College of pharmacy and Research centre, Trivandrum.

ACUTE TOXICITY STUDY

Acute toxicity study of methanolic extract of the aerial parts of *Pavetta indica* was determined in male Wistar albino rats according to OECD guidelines No. 425[10]. The overnight fasted animals were orally administered with the methanolic extract 2000 mg/kg body weight. Animals were continuously monitored for first 1 h and then hourly for 4 hours then after every 24 hours for 14 days for death, signs of discomfort, general behaviour and nervous manifestations. Pre-screening examination with 1/20th, 1/10th and 1/5th of 2000 mg/kg, i.e. 100, 200 and 400 mg was done. The doses 200 and 400 mg/kg were selected for hepatoprotective study.

INDUCTION OF CARBON TETRA CHLORIDE HEPATOTOXICITY AND EXPERIMENTAL DESIGN[11]

For the study animals were divided into 5 groups and each group contained 6 rats of male sex.

Group I

Served as normal control and was administered with 10% acacia suspension (1 ml/kg, p.o) daily for 5 days with olive oil (1 ml/kg, i.p) on days 2 and 3.

Group II

Served as negative control and was administered with 10% acacia suspension (1 ml/kg, p.o) daily for 5 days along with CCl_4: olive oil (1:1, 2 ml/kg, i.p) on days 2 and 3.

Group III

Treated with reference drug Silymarin (50 mg/kg, i.p) along with 10% acacia (1 ml/kg, p.o) daily for 5 days along with CCl_4: olive oil (1:1, 2 ml/kg, i.p) on days 2 and 3, 30 mins. After administration of reference drug.

Group IV

Treated with methanolic extract of the aerial parts of the plant, *Pavetta indica* (200mg/kg) along with 10% acacia (1 ml/kg, p.o) daily for 5 days with CCl_4: olive oil (1:1, 2 ml/kg, i.p) on days 2 and 3, 30mins. After administration of test dose.

Group V

Treated with methanolic extract of the aerial parts of the plant, *Pavetta indica* (400mg/kg) along with 10% acacia (1 ml/kg, p.o) daily for 5 days with CCl_4: olive oil (1:1, 2 ml/kg, i.p) on days 2 and 3, 30mins. After administration of test dose.

During the period of treatment the rats were maintained under normal diet and water. On the 5th day the overnight fasted rats were humanely sacrificed by using CPCSEA recommended euthanasia procedure (Carbon dioxide inhalation method).

BIOCHEMICAL ASSAY

Blood samples were collected by intra cardiac puncture and liver enzymes were analysed. SGOT (Aspartate transaminase), SGPT (Alanine transaminase), Alkaline Phosphatase (ALP) and TB (Total Bilirubin) estimation were carried out using commercial kits. Histopathological assessment of liver damage was also studied.

STATISTICAL ANALYSIS

All values were expressed as Mean ±SEM. The data was statistically analyzed by Bonferroni Multiple Comparison Test. P value less than 0.0001 was considered as statistically significant.

RESULTS AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary investigation on the different extracts of aerial parts of *Pavetta indica* disclose that the methanolic extract contains chemical constituents of pharmacological importance such as alkaloids, flavonoids, saponins and phenolic compounds which is shown in Table No.1.

TOTAL ANTI OXIDANT ACTIVITY BY PHOSPHOMOLYBDENUM METHOD

The total antioxidant assay is based on reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V). The incubation of extracts with the Molybdenum (VI) will show the presence of antioxidant components in the extract, that is the formation of reduced green molybdenum complex[12]. Thus this method is very useful to determine the antioxidant activity of crude extracts on the total basis**.** Among the different extracts used for the study, the methanolic extract of aerial parts of *Pavetta indica* was found to have a promising anti-oxidant activity. Studies have proved that the carbonyl groups present in the flavanoids and phenolic compounds are responsible for antioxidant activity[13,14]. The presence of these phytoconstituents in the methanolic extract may be accountable for the promising antioxidant activity. The results are shown in Table No.2.

HEPATOPROTECTIVE ACTIVITY

ACUTE TOXICITY STUDY

Acute toxicity studies showed that the methanolic extract of aerial parts of *Pavetta indica* was found to be safe at a dose upto 2000mg/kg body weight as it did not show any mortality.

***IN VITRO* STUDY**

In vitro hepatoprotective activity was studied for methanolic extract of aerial parts of *Pavetta indica herb* by MTT assay method using Chang liver cell line. It was observed that with the increase in concentration of the extract the percentage of viable cells also increases. Thus it was proved that the extract has the ability to preserve the structural integrity of hepatocellular membrane and the results are given in Table No.3.

***IN VIVO* STUDY**

The results unveil that hepatotoxicity induced animal models showed a significantly high levels of hepato specific enzymes- Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT,) Alkaline Phosphatase (ALP) and total bilirubin (TB) when compared to control group which indicate acute hepatocellular damage and biliary obstruction. The rats treated with standard drug silymarin and methanolic extract (200 and 400mg/kg body weight) exhibit a decrease in the elevated levels of these parameters. Decrease in the levels of SGPT, SGOT, ALP and TB was an evidence of plasma membrane stabilization as well as repair of

damaged hepatic tissue caused by Carbon tetra chloride. This emphasize that the methanolic extract of aerial parts of *Pavetta indica* herb has a significant hepatoprotective activity, the results are given in Table No.4. Histopathological evaluations of liver sections are shown in Figure No.4.

Reactive oxygen species (ROS) and free radicals result in cellular aging which occurs through the destabilization of membranes in cells. These free radicals possibly oxidize nucleic acids, proteins, lipids or DNA and cause degenerative disease. Many physiological processes and diseases, like Cancer, Arthritis, Parkinson's syndrome, Ischemia and Liver injury occurs due to the involvement of highly reactive molecules -Free radicals and ROS. During liver damage there is increase in the level of free radicals and decreased scavenging potential of cells.

The main feature of an antioxidant is its potential to trap free radicals[13]. Studies have proved that the flavanoids and phenolic compounds play an important role in reducing the oxidative stress produced by reactive oxygen species and hydroxyl radicals generated in liver diseases[15]. Along with flavanoids and phenolic compounds literature also proves that alkaloid related compounds also has good potential for treating liver diseases[16]. The presence of these phytoconstituents and significant antioxidant potential of the methanolic extract of aerial parts of *Pavetta indica* may be playing a major role in stimulating the hepatoprotective activity. Thus the *in vitro* and histopathological studies support the hepatoprotective activity of the extract.

Table No.1: Preliminary phytochemical screening

S.No	Phytoconstituents	PEEP	CEP	EAEP	MEP
1	Alkaloids	-	-	+	+
2	Carbohydrates	+	+	+	+
3	Protein	+	+	-	-
4	Flavanoids	-	+	-	+
5	Saponin	+	+	+	+
6	Glycoside	+	-	-	-
7	Phenolic	-	+	-	+

PEEP-Pet. ether extract of *Pavetta indica*, CEP- chloroform extract of *Pavetta indica*, EAEP- Ethyl acetate extract of *Pavetta indica*, MEP- Methanolic extract of *Pavetta indica*

Table No.2: Total antioxidant activity by phosphor molybdenum method

S.No	Sample	OD at 695nm	Con.of antioxidant in µg/mg of sample
1	PEEP	0.556	69.5
2	CEP	0.351	43.87
3	EAEP	0.881	110
4	MEP	1.638	204.75

PEEP-Pet. ether extract of *Pavetta indica*, CEP- chloroform extract of *Pavetta indica*, EAEP- Ethyl acetate extract of *Pavetta indica*, MEP- Methanolic extract of *Pavetta indica*

Table No.3: Percentage of viable cells in different concentrations of MEP by MTT assay method

S.No	MEP(µg/ml)	% of viable cells
1	Control	100
2	10	53.06 ± 0.61 *
3	20	63.61± 0.30 *
4	40	72.44 ± 1.33 *
5	80	80.18 ± 0.43 *
6	100	89.92 ± 0.59 *

MEP-methanolic extract of Pavetta indica herb, Data is expressed as mean±SE, n=3. *P<0.05 when compared with control.

Table No.4: Hepatoprotective Activity of MEP

S.No	Group	SGOT(IU/L)	SGPT (IU/L)	ALP(IU/L)	TB(mg/dl)
1	Control	139±0.53	281 ± 1.9	154±0.125	1.25±0.025
2	Negative	303±1.1	305 ± 1.0	395±0.042	3.54±0.035
3	Standard	184±0.36	65 ± 1.1	222±0.158	2.16±0.022
4	Extract(200mg)	243±0.43*	199 ± 1.6*	324±0.146*	2.84±0.147*
5	Extract(400mg)	206±0.38*	100 ± 0.99*	301±0.947*	2.57±0.051*

Data is expressed as mean ± SE, n=6 in each group. MEP- methanolic extract of *Pavetta indica* herb, *P<0.0001 when compared to standard.

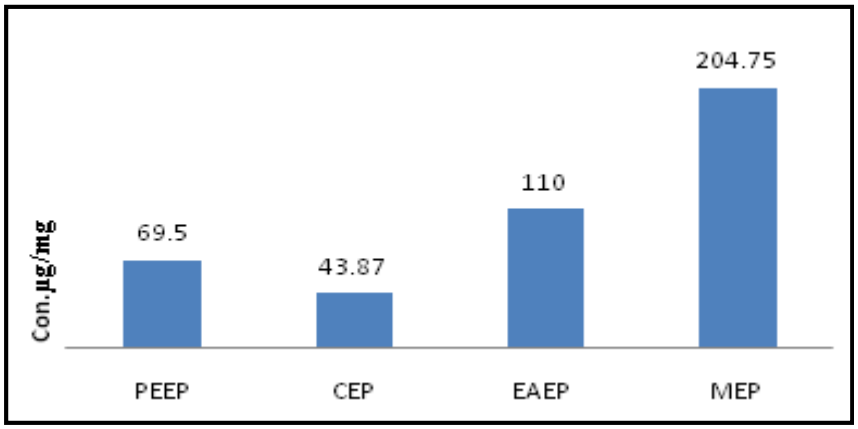

Figure No.1: Total antioxidant levels of different extracts of *Pavetta indica* herb

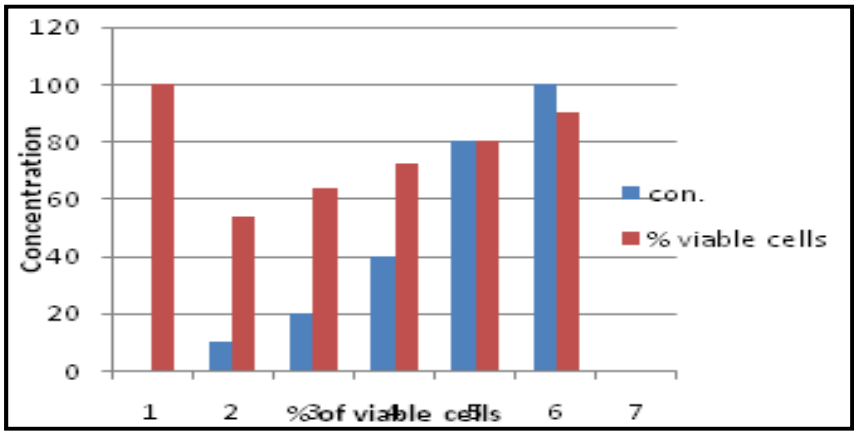

Figure No.2: Relationship between different concentrations of MEP and % of viable cells

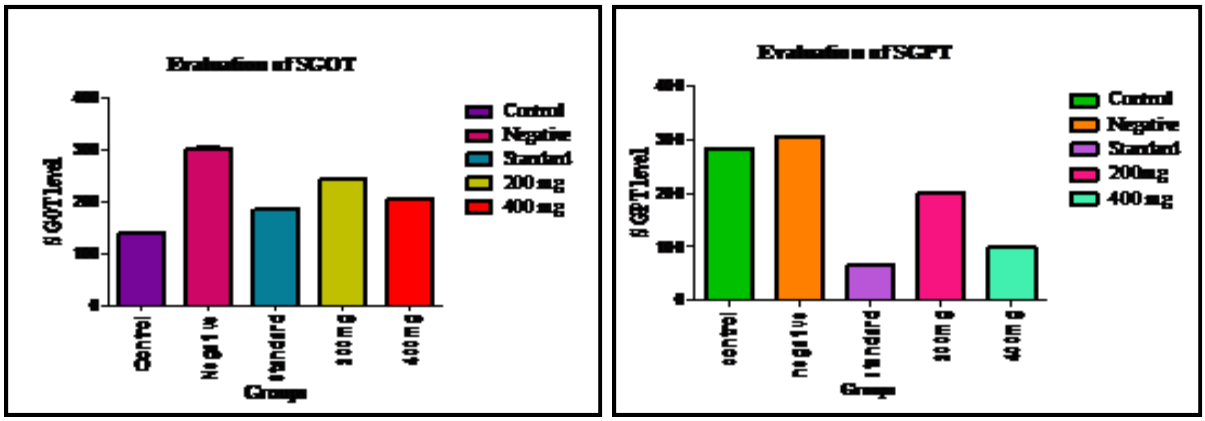

Figure No.3: Evaluation of SGPT and SGOT on CCl4 induced rats

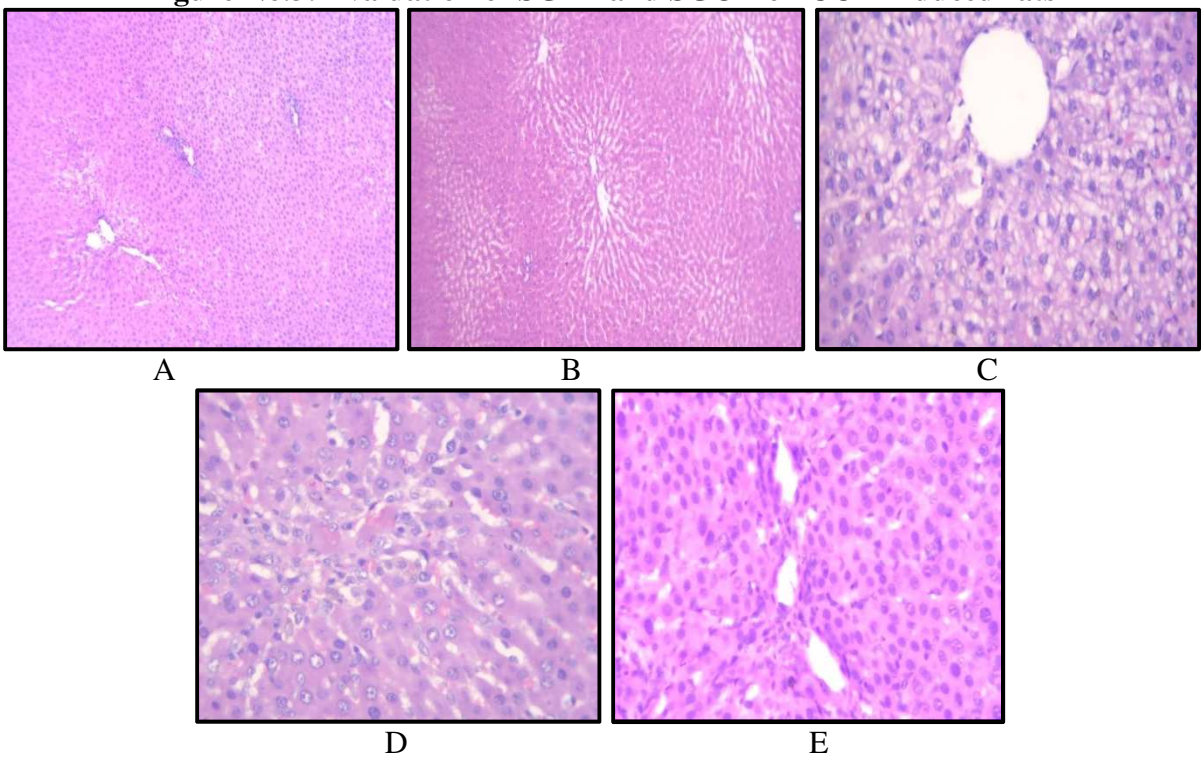

Figure No.4: Histopathological evaluation of hepatoprotective activity of MEP

A- Liver section of control group, B- CCl4 induced hepatotoxicity, C- CCl4+ Silymarin (50mg/kg body weight), D- CCl4+ MEP (200mg/kg body weight), E- CCl4+ MEP (400mg/kg body weight), MEP- methanolic extract of *Pavetta indica*

CONCLUSION

In conclusion, the result of study indicates that the methanolic extract of *Pavetta indica herb* owns hepatoprotective property. This property may be promoted due to the antioxidant activity and also owing to the presence of phytoconstituents like flavanoids and phenolic compounds in the extract. Further studies are required to identify, isolate, characterize, and evaluate the active principle responsible for hepatoprotective activity of plant. Study on the isolation and characterization of the hepatoprotective principle is in progress. Further studies regarding the exact mechanism of action of the active compound for the hepatoprotective effect is need to be interpreted.

ACKNOWLEDGMENT

For the excellent and helpful support to enable the successful completion of this work, the authors are thankful to Athmic Biotec solutions Pvt. Limited, at kalliyoor, Kerala, India. For presenting the opportunities to carry out the hepatoprotective *in vivo* study the authors are indebtly thankful to The Dale View College of Pharmacy and Research Centre, Trivandrum, India.

CONFLICT OF INTEREST

Nil

BIBLIOGRAPHY

1. Eduardo Madrigal-Santillán *et al.* Review of natural products with hepatoprotective effects, *World J Gastroenterol,* 20(40), 2014, 14787-14804.
2. Handa S S, Sharma A. Hepatoprotective activity of Andrographolide from Andrographis paniculate against CCI4, *Indian J Med Res,* 92, 1990, 276-283.
3. Weber L W, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, *Crit Rev Toxicol,* 33(2), 2003, 105-36.
4. Ahmed Wahid, Ashraf Hamed N, Heba Eltahir M and Mekky Abouzied M. Hepatoprotective activity of ethanolic extract of *Salix subserrata* against CCl_4-induced chronic hepatotoxicity in rats, *BMC Complementary and Alternative Medicine,* 16(1), 2016, 263.
5. Zhi-Qiang Huang, Pan Chen, Wei-Wei Su, Yong-Gang Wang, Hao Wu, Wei Peng and Pei-Bo Li. Antioxidant Activity and Hepatoprotective Potential of Quercetin 7-Rhamnoside *In Vitro* and *In Vivo*, *Molecules,* 23(5), 2018, 1188.
6. John Peter Paul J, Yuvaraj P, Iniya Udhaya C. Hepatoprotective Potential of Methanolic Extract of *Lobophora Variegata* (Lamour.) Womersley Ex Oliviera, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 6(4), 2018, 188-193.
7. Trease G E and Evans M C. Text book of Pharmacognosy, *London: Bailier Tindall,* 12th Edition, 2002, 343-382.
8. Harborne J B. Phytochemical methods, A guide to modern techniques of plant analysis, *Springer India,* 3rd Edition, 1998, 49-120.
9. Prieto P, Pineda M and Anguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdaenum complex: specific application to the determination of vitamin E, *Analytical Biochemistry,* 269(2), 1999, 337-341.
10. OECD, Guidelines for testing of chemicals, Acute oral toxicity, Environmental Health and Safety Monograph Series on Testing and Adjustment No. 425, 2001, 1.
11. Bharathi R, Ravi Sankar K, Geetha K. *In vitro* antioxidant activity and *in vivo* hepatoprotective activity of ethanolic whole plant extract of *Nymphoides hydrophylla* in ccl4 induced liver damage in albino rats, *Int.J.Res.Ayurveda Pharm,* 5(6), 2014, 667-72.
12. Narayanan Ravisankar, Chandrasekaran Sivaraj, Sooriamuthu Seeni, Jerrine Joseph, Nanjian Raaman. Antioxidant Activites and Phytochemical Analysis of Methanol Extract of Leaves of Hypericum Hookerianum, *Int J Pharm Pharm Sci,* 6(4), 2014, 456-460.
13. Varsha Sharma, Agrawal C. *In vivo* antioxidant and hepatoprotective potential of Glycyrrhiza glabra extract on carbon tetra chloride (CCl4) induced oxidative-stress mediated hepatotoxicity, *Int J Res Med Sci,* 2(1), 2014, 314-320.
14. Reza Azadi Gonbad, Adlin Afzan, Ehsan Karimi, Uma Rani Sinniah, Mallappa Kumara Swamy. Phytoconstituents and antioxidant properties among commercial tea (Camellia sinensisL.) clones of Iran, *Electronic Journal of Biotechnology,* 18(6), 2015, 433-438.
15. Swaroopa Rani Vanapatla, Sana Syed, Praneetha Pallerla, Ravi Kumar Bobbala.

Antioxidant and Hepatoprotective Studies on Methanolic Extract of Caryopses of Echinochloa frumentacea Link, *IJPPR,* 9(10), 2017, 1386-1391.

16. Padmalochana K, Dhana Rajan M S, Lalitha R, Sivasankari H. Evaluation of the Antioxidant and Hepatoprotective Activity of Cryptolepis Buchanani, *Journal of Applied Pharmaceutical Science,* 3(02), 2013, 099-104.
17. Shahidi F, Zhong Y. Lipid oxidation and improving the oxidative stability, *Chem Soc Rev,* 39(11), 2010, 4067-4079.
18. Sarian M N *et al.* Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship based study, *Biomed. Res. Int,* Article ID 8386065, 2017, 14.
19. Seyoum A, Asres K, El-Fiky F K. Structure-radical scavenging activity relationships of flavonoids, *Phytochemistry,* 67(18), 2006, 2058-2070.
20. Heim K E, Tagliaferro A R, Bobilya D J. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships, *J. Nutr. Biochem,* 13(10), 2002, 572-584.
21. Kingshuk Lahon and Swarnamoni Das. Hepatoprotective activity of *Ocimum sanctum* alcoholic leaf extract against paracetamol-induced liver damage in Albino rats, *Pharmacognosy Res,* 3(1), 2011, 13-18.
22. Jafri M A, Jalis S, Kalim J M, Surender S. Hepatoprotective activity of leaves of Cassia occidentalis against paracetamol and ethylalchohol intoxication in rats, *Journal of Ethnopharmacology,* 66(3), 1999, 355-361.
23. Jain S, Jain D K, Balekar N. *In Vivo* Antioxidant activity of ethanolic extract of Mentha pulegium leaf against CCl4 induced toxicity in rats, *Asian Pacific Journal of Tropical Biomedicine,* 2(2), 2012, S737-S740.
24. Sandhir R, Gill K D. Hepatoprotective effects of Liv52 on ethanol induced liver damage in rats, *Indian J Expt Biol,* 37(8), 1999, 762-68.

Please cite this article in press as: Seena H *et al.* Evaluation of phytochemical, anti-oxidant and hepatoprotective activities of methanolic extract of *pavetta indica*, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 7(2), 2019, 379-387.