Research Article

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

ISSN	Print	2231 – 3648
	Online	2231 – 3656

PHYTOCHEMICAL SCREENING AND *IN VITRO* HEPATOPROTECTIVE ACTIVITY ON THE BERRIES OF *VITEX AGNUS-CASTUS*

*Jennifer Margaret J, Jayshree N

Department of Pharmacognosy, College of Pharmacy,
Madras Medical College, Chennai-600 003, T.N, India.

Abstract

Liver, the most vital organ present in the human body, plays a major role in metabolism and excretion of xenobiotics and is vulnerable to a wide variety of metabolic, circulatory and neoplastic insults. Modern medicine offers limited success in providing effective cure and there is a need to develop herbal drugs capable of healing toxic liver damages. *Vitex agnus-castus* (Verbenaceae), generally called as the Women's herb, has a folklore claim of treating liver disorders. In the present study preliminary phytochemical screening and *in vitro* hepatoprotective studies of *Vitex agnus-castus* were performed. The phytochemical studies showed the presence of secondary metabolites such as flavonoids, glycosides, alkaloids, saponin, phytosterol and phenolic compounds. The *in vitro* hepatoprotective activity on the berries of *Vitex agnus-castus* was carried out using different extracts by MTT assay using normal Chang liver cell line by paracetamol induced hepatotoxicity method. Ethyl acetate extract showed high protection against paracetamol induced hepatotoxicity and was found to be the best extract when compared to the other extracts.

Keywords: Cytotoxicity, Hepatoprotective, *Vitex agnus-castus*, Paracetmol.

Introduction

Liver, the most vital organ present in the human body plays a major role in metabolism and excretion of xenobiotics and is vulnerable to a wide variety of metabolic, circulatory and neoplastic insults.[1] Hepatotoxicity is a term which is defined as an injury to the liver that is associated with impaired liver function caused by exposure to a drug or other non-infectious agents. When function of liver is impaired, it is characterized by altered liver function tests and clinically significant disease follows. Drug related hepatotoxicity is the one which is life threatening and /or requires hospitalization in serious cases. Studies report that the incidence of drug induced hepatotoxicity is 1 in 10,000.[2] In most cases, there is no effective treatment other than stopping the drug and providing general care. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market.[3]

The traditional medicine refers to a broad range of ancient and natural health care practices including tribal practices as well as Ayurveda, Siddha and Unani.[4] These have been used for treating many health complications and also liver disorders. It is

Author for Correspondence:
Jennifer Margaret J,
Department of Pharmacognosy, College of Pharmacy,
Madras Medical College, Chennai-600 003, T.N, India.
E-mail: jenniferamali@gmail.com

estimated that more than 7500 plants are used mostly in rural and tribal villages of India.[5]

Vitex agnus-castus (Verbenaceae), commonly known as hemp tree and monk's pepper, is used as a complementary medicine in Europe.[6] It has been used therapeutically in treating premenstrual syndromes, mastalgia, hormonal disorders, menstrual cramps, dropsy and cancer. In the present study phytochemical screening and *in vitro* hepatoprotective activity on the berries of *Vitex agnus-castus* was carried out.[7]

Materials and methods

Plant collection and extraction procedure

The berries were collected from Rajavallipuram, Thirunelveli District, Tamil Nadu, India and it was botanically identified and authenticated by Dr. V. Chelladurai, Research Officer- Botany (Scientist – C), Central Council for Research in Ayurveda and Siddha, Government of India. The dried coarsely powdered berries of *Vitex agnus-castus* were extracted using Soxhlet apparatus with solvents of increasing polarity such as Hexane, Ethyl acetate and Ethanol at 60-70˚C for of 18 hours. All the extracts were redistilled and concentrated under rotary vacuum evaporator were tested for preliminary phytochemical screening.[8]

Cell line maintenance and culture

Normal Chang liver cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO_2 at 37˚C. Cultures were maintained by weekly passage and the culture medium was changed twice a week.

Cytotoxicity screening by tetrazolium (MTT) assay[9]

- The Chang liver monolayer cells were detached with Trypsin-ethylene Diamine tetra acetic acid (EDTA) to make single cell suspensions and the viable cells were counted using a haemocytometer and diluted with medium along with 5% FBS to give final density of 1×10^5cells/ml.
- Cells (1×10^5/ well) were plated in 5ml of medium/well in 96 well plates (Coster Corning, Rochester, NY).
- After 48 hours incubation, the cell reaches the confluence. Then, cells were incubated with different concentrations (1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8µg/ml) of Silymarin, Hexane, Ethylacetate and Ethanol for 24-48hrs at 37˚C.
- After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 1ml/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added.
- After 4 hours incubation, 0.04M HCl/ isopropanol were added.
- Viability of cells was determined by measuring the absorbance at 570nm using UV spectrophotometer and wells not containing sample were treated as blank.
- Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically.
- Triplicate was maintained for all concentrations.
- The effect of the samples on the proliferation of Chang Liver cells was expressed as the % cell viability, using the following formula,

$$\% \text{ cell viability} = \frac{\text{A 570 of treated cells}}{\text{A 570 of control cells}} \times 100$$

In vitro hepatoprotective activity using various extracts against paracetamol induced toxicity [10]

The same procedure as given above was repeated. The only additional step is inclusion of challenging the confluent monolayer cells with Paracetamol (125µg/ml) and then incubating with the various concentrations of extracts of berries.

Results

Preliminary phytochemical analysis of the various extracts of the berries of *Vitex agnus-castus* showed that the ethyl acetate extract possessed maximum phytoconstituents such as carbohydrates, flavonoids, glycosides, alkaloids, saponins, phenolic compounds, tannins and triterpenoids. (Table 1)

The standard silymarin and different extracts of the berries of *Vitex agnus-castus* were screened for their cytotoxicity effect on the Chang liver cell line over different concentration ranges of 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8µg/ml. The 50% cell

viability (CTC_{50}) of all the extracts was found to be 62.5µg/ml which is same as that of silymarin. Hence it is proved that the extracts are non toxic against the cell line. (Table 2)

To test the hepatoprotective potential of the *Vitex agnus-castus,* the Chang liver cells were first challenged with paracetamol (125µg/ml) and then treated with silymarin and the various extracts in concentrations of 100, 50, 25, 10µg/ml. The cell viability of the paracetamol alone challenged group was 39.92%. In the group challenged with paracetamol and treated with the standard drug silymarin, there was a concentration dependent increase in cell viability. At the highest concentration (100µg/ml), the cell viability was 73.1%. (Table 3)

For the different extracts tested, the ethyl acetate extract showed a remarkable increase in cell viability and it was 72.9% at the concentration of 100µg/ml. This is almost equal to the standard drug silymarin. The other extracts (ethanol and hexane) also showed an improvement in cell viability, though not as high as ethyl acetate.

Discussion

The berries of *Vitex agnus-castus* have a folklore claim of being useful in hepatic disorders and also used as complementary medicine in Europe. There are no studies to scientifically support this. Hence the present study was carried out. The phytochemical studies showed that among various extracts, the ethyl acetate extract showed the presence of flavonoids, glycosides, alkaloids, saponins, phenolic compounds, tannins and triterpenoids. The presence flavonoids, glycosides and alkaloids contribute to hepatoprotective activity in many plants. The presence of flavonoids such as quercetin in *Phyllanthus emblica*[11], luteolin, apigenin in *Equisetum arvense*[12], citromitin, tangeretin in *Citrus depressa*[13], glycosides such as acubin from *Plantago asiatica,* picroside I and II from *Picorrhiza kurroa*[14] and alkaloids such as steroidal alkaloids in *Hygrophila auriculata*[15] showed potent liver protecting activity. In this study, the berries of *Vitex agnus*-castus have been shown to be rich in flavonoids, glycosides and alkaloids. Hence, the hepatoprotective activity of *Vitex agnus-castus* may be due to the presence of these phytoconstituents.

Toxicity studies on normal Chang liver cell line showed that all the extracts were non-toxic. The *in vitro* hepatoprotective studies using paracetamol induced hepatotoxicity on Chang liver cell line showed that the ethyl acetate extract offered maximum protection against hepatotoxicity caused by paracetamol. The cell viability in the ethylacetate and paracetamol treated group was 72.9% as against the cell viability of paracetamol treated group which was 39.92%.

Hence from this study we conclude that the ethyl acetate extract of the berries of *Vitex agnus-castus* are rich in several phytoconstituents and they show good hepatoprotective activity which is comparable with that of the standard drug silymarin. Further *in vivo* studies will help in confirming this activity.

Table No. 01: Phytochemical analysis of different extracts of *Vitex agnus-castus*

S.No.	Phytoconstituents	Hexane	Ethyl Acetate	Ethanol
1.	Flavonoid	-	+	+
2.	Glycosides	-	+	-
3.	Alkaloids	-	+	+
4.	Saponin	-	+	-
5.	Phytosterols	+	-	-
6.	Phenolic compounds	-	+	+
7.	Proteins	-	-	-
8.	Fixed oils and fats	-	-	-
9.	Tannins	-	+	-
10.	Triterpenoids	+	+	+

+ indicates presence - indicates absence

Table No. 02: Determination of cytotoxicity effect by using Normal Chang liver cell line

S.No.	Concentration (µg/ml)	% cell viability			
		Silymarin	Hexane	Ethyl acetate	Hexane
1.	1000	6.77	15.25	11.86	8.47
2.	500	18..64	25.42	22.03	18.64
3.	250	32.20	35.59	33.89	32.20
4.	125	40.67	42.37	44.06	40.67
5.	62.5	51.84	47.45	50.84	48.15
6.	31.2	69.49	54.23	62.71	57.62
7.	15.6	79.66	62.71	76.27	71.18
8.	7.8	89.83	69.49	88.13	83.05
9.	Cell control	100	100	100	100

Table No. 03: Protective effect of the given extracts on paracetamol induced toxicity in Chang liver cells

S.No.	Treatment	Concentration µg/ml	% cell viability
1.	Control		100
2.	Paracetamol	125	39.92±1.02
3.	Paracetamol + silymarin	10	55.9±1.15
		25	60.4±1.04
		50	65.8±1.43
		100	73.1±1.09
4.	Paracetamol + ethyl acetate	10	49.8±1.24
		25	58.3±1.31
		50	62.5±1.57
		100	72.9±1.43
5.	Paracetamol + ethanol	10	36.6±1.83
		25	47.9±1.26
		50	52.5±1.74
		100	58.7±1.89
6.	Paracetamol + hexane	10	32.6±1.55
		25	41.3±1.21
		50	49.2±1.92
		100	57.1±1.34

Values are expressed as Mean ± S.E.M.

Reference

1. Robbins and Cotran. Pathological basis of Diseases. 8th edition. Elsevier publishers. 2013, 834.
2. Victor J. Navarro, M.D., John R. Senior, M.D. Drug related Hepatotoxicity. The New England Journal of Medicine., 354, 2006, 731-9.
3. Larrey D. Epidemiology and individual susceptibility to adverse drug reactions affecting the liver. Semin Liver Dis., 22, 2002, 145-55.
4. G. D. Chardhary, P. Kamboj, I. Singh, A. N. Kalia. Herbs as liver savers – A review. Indian Journal of Natural products and resources., 1(4), 2010, 397-408.
5. Sanjoy Kumar Pal, Yogeshwer Shukla. Herbal medicine: Current status and the future Asian Pacific Journal of Cancer Prevention., 4, 2003, 281- 288.
6. Dr. K. M. Nadkarni. The Indian Materia Medica. 3rd edition vol 1, Bombay: Popular book depot., 2009, p.1277.
7. C. P. Khare. Indian Medicinal Plants. Springer publications, 2007. p. 709.
8. Harbone JB. Phytochemical methods. London: Chapman and Hall Ltd., 1973.p.49-188.
9. Mosmann T. Rapid Colorimetric assay for Cellular growth and Survival: Application to Proliferation and Cytotoxicity assays. Journal of Immunological Methods., 65, 1983, p. 55-63.
10. Mahendrajain *et al*. Cytotoxicity evaluation and hepatoprotective potential of bioassay guided fractions from the leaves of *Feronia limmonia* Linn. Asian Pacific Journal of Tropical Biomedicine., 2011, 443-447.
11. Gulati RK, Agarvwal S, Agarwal SS. Hepatoprotective studies on *Phyllanthus*

emblica Linn. And quercetin. Indian Journal of Experimental biology., 33, 1995, 261-8.

12. Oh H, Kim DH, Cho JH, Kim YC. Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from *Equisetum arvense*. Journal of Ethnopharmacol., 95, 2004, 421-4.
13. Akachi T *et al.* Hepatoprotective effects of flavonoids from shekwasha (*Citrus depressa)* against D-galactosamine induced liver injury in rats. Journal of nutritional science and vitaminology., 56, 2010, 60-7.
14. M. F. Valan *et al.* Phytoconstituents with hepatoprotective activity. International journal of chemical science., 8(3), 2010, 1421-1432.
15. Raj VP *et al. In vitro* and *in vivo* hepatoprotective effects of the total alkaloid fraction of *Hygrophila auriculatan leaves.* Indian Journal of Pharma col., 42(2), 2010, 99-104.