IJBPAS, March, 2020, 9(3): 575-589

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ASSESSMENT OF HEPATO-SHIELDING AND ANTI-INFLAMMATORY PROPERTIES OF VARIOUS EXCERPTS OF *Pteris vittata*

WAHEED I[1], RANA A[1] AND SALEEM F[1], ASHRAF N[1] AND WAQAS M[2*]

1: Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan

2: School of Pharmaceutical Sciences, Johar Institute of Professional Studies, Lahore, Pakistan

[*]**Corresponding Author, Muhammad Waqas (PhD): waqaskhanjips@gmail.com; waqas@jips.edu.pk; +923213535005**

Received 10th Oct. 2019; Revised 8th Nov. 2019; Accepted 9th Dec. 2019; Available online 1st March 2020

https://doi.org/10.31032/IJBPAS/2020/9.3.5015

ABSTRACT

Pteris vittata has traditionally been used for wound healing, abdominal pains, diarrhea but its hepato-protective and anti-inflammatory activities still cause of concern. Three extracts were prepared i.e., n-hexane, chloroform and methanol by using successive extraction. Hepatoprotective activity of these were checked in rats by carbon tetrachloride (CCl_4 0.5 ml/kg) induced hepatic insult and Silymarin 200 mg/kg was used as standard drug. The biochemical and histopathological parameters were determined. I*n-vitro* anti-inflammatory activity was done by using egg albumin denaturation method. The biochemical parameter confirmed that methanol extract of *Pteris vittata* lowers the alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and cholesterol levels in rats and has shown maximum hepatoprotective activity as compared to other extracts. The histopathological examination of liver tissues also indicated that the methanol extract of *Pteris vittata* exhibited the strongest hepatoprotective activity against CCl_4 induced hepatotoxicity. *In-vitro* anti-inflammatory activity showed that the value of IC_{50} confirmed that chloroform and n-hexane extract exhibited maximum anti-inflammatory activity when compared to standard drug (diclofenac sodium).

It is concluded that *Pteris vittata* possesses hepatoprotective and anti-inflammatory activities which might be due to the presence of phytoconstituents which were confirmed by phytochemical investigation. *Pteris vittata* if used as natural remedy will help the mankind to recover from illness like liver diseases and inflammation without any significant adverse effect.

Keywords: *Pteris vittata*, hepatoprotective, carbon tetrachloride, anti-inflammatory

1. INTRODUCTION

Liver plays an important role in survival as it is involved in the coordination of metabolism in the body which includes xenobiotic metabolism, glucose homeostasis and removal of toxicants. Synthesis of steroidal hormones and plasma proteins degradation and synthesis take place in liver. Through systemic supply of nutrients energy is continuously provided to the whole body, which is managed by the liver **[1-2]**. Particularly liver is susceptible to injuries induced by chemicals. The chemical induced liver diseases have a wide spectrum, ranging in severity from minor variations in liver structure to acute hepatic failure, cirrhosis and cancer of liver **[3]**. The ability to prevent liver damage is called anti-hepatoxicity or hepatoprotection. The protective response of the body to damaging stimuli such as infectious microorganisms and irritants/allergens is known as inflammation. Molecular mediators, damage to blood vessels and immune cells are the major cause of inflammation **[4]**. Most important mechanism involved in inflammation is metabolism of arachidonic acid (AA). AA is metabolized by two pathways named cyclooxygenase (COX) pathway and 5-lipoxygenase pathway (5-LOX) to prostaglandins (PG), thromboxane A2 and to hydroperoxy-eicosatetraenoic acid (HPETE'S), leukotrienes (LT) respectively. These all mediators are essential in series of inflammatory events. AA can cross the endothelium membrane due to neutrophils stimulation and as a result of this, COX and 5-LOX pathway can convert AA to prostaglandins and leukotrienes. A drug that causes inhibition of COX and 5-LOX pathways resulting in decreased production of LT and PG would have the great anti-inflammatory and analgesic effect and have fewer side effects on gastrointestinal tract (GIT) **[5]**. Since the beginning of human civilization medicinal plants have been used because of their therapeutic values and a large number of allopathic/modern drugs are isolated from natural sources **[6]**. To

fulfill primary health care needs about 80% of the world's population depends mainly on natural medicines **[7]**. The presence of various secondary metabolites in plants are responsible for their medicinal properties. These secondary metabolites have a wide range of bio medicinal activity, safety and efficacy in order to obtain a variety of drugs from them **[4]**. *Pteris vittata* is a pteridophyte belonging to the Pteridaceae family. *Pteris vittata* is native to China but also cultivated in many countries **[8]**. It is commonly growing along roadsides and calcareous substrates such as in habitats with high alkaline pH and places contaminated with arsenic **[9]**. It is universally known as hyper accumulator of arsenic. Traditionally, *Pteris vittata* has been used in the treatment of abdominal pain, diarrhea and flu. Relatively less information is present in literature regarding the pharmacological activities of ferns **[10]**. *Pteris vittata* is reported to haveanti-oxidant properties,anti-cancer properties, anti-microbial properties and anti-diabetic properties **[11-12]**. In current study *in-vivo* hepatoprotective (rats as animal model) and *in-vitro* anti-inflammatory activities of different extracts of *Pteris vittata* were evaluated.

2. MATERIALS AND METHODS

2.1. Animals: Swiss albino rats having weight 150-200 gm were used in this study and were kept under environmentally controlled conditions i.e. 25±2 ºC. The 12 h dark/light cycle was maintained and they were fed with standard diet. The experiment protocol was accepted by the Institutional Research Ethics Committee (IREC) as described in The University of Lahore guidelines.

2.2. Chemicals: Carbon tetrachloride, silymarin, solvents for extractions and phytochemical analysis were procured from department laboratory

2.3. Plant material and solvent extraction: *Pteris vittata* was collected from botanical garden of Government College University, Lahore. The whole plant was shade dried and then pulverized. The powdered plant material (1 kg) was successively extracted by using n-hexane, chloroform and methanol. The powdered plant material was soaked in n-hexane, chloroform and methanol for 7 days each and then filtered. To obtain extracts these three filtrates were subjected to rotary evaporator (Heidolph Laborota 4000 Rotavap, Germany) under reduced pressure.

2.4. Hepatoprotective activity study design and treatments:

On the basis of previously reported studies experimental protocol was followed **[13]**. Six groups were made having five rats in each group. Rats of group 1 were given only vehicle 1 ml/kg/day of 1% carboxy-methylcellulose (CMC); per os (p.o.) for

seven days. Rats of group 2 were treated with 0.5 ml/kg dose of CCl_4 i.p. once daily for seven days. Rats of group 3 received 0.5 ml/kg dose of CCl_4 i.p. and 200 mg/kg dose of silymarin orally for seven days. Rats of group 4 to 6 were given i.p. 300 mg/kg dose of n-hexane, chloroform and methanol extracts of *Pteris vittata* respectively and 0.5 ml/kg dose of CCl_4 for seven days.

After 24 h of last treatment, animals were sacrificed in order to collect their blood from heart. **2.4.1. Biochemical analysis:** Biochemical analysis was done by separating the blood serum at 2500 rpm for 15 min. Liverfunction biochemical markers (ALT, AST, total bilirubin and cholesterol) were checked in the blood serum of rats. ALT and AST levels were measured using spectrophotometric analysis **[14]**. Total bilirubin levels were determined using method proposed by Walters **[15]**. Levels of total cholesterol were determined using method proposed by Allain **[16]**.

2.4.2. Histopathological analysis: Liver samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) for general histopathological analysis. The slides were observed under light microscope and the liver histology from different treatment groups were compared.

2.5. In-vitro anti-inflammatory activity/Inhibition of albumin denaturation: On the basis of previously reported studies experimental protocol was followed **[17]**. Double distilled water was used as control. Samples were prepared by making different concentrations (25, 50,100, 250, 500, 800 and 1000 µg/L) of standard drug (Diclofenac sodium) and three extracts of *Pteris vittata.* Diclofenac sodium was used as standard drug. Standard solution is prepared by adding 0.2 ml of egg albumin, 2.8 ml of phosphate buffer saline and 2 ml of different concentrations (25, 50, 100, 250, 500, 800 and 1000 µg/L) of diclofenac sodium separately. It was prepared by adding 0.2 ml of egg albumin, 2.8 ml phosphate buffer saline and 2 ml of all the three extracts of *Pteris vittata* of different concentrations (25, 50, 100, 250, 500, 800 and 1000 µg/L) separately. Sample mixture was incubated at 37 ºC for 15 min. After that sample mixture was heated at 70 ºC in water bath for 5 min. and cooled down. The sample solution was checked for the presence of turbidity and if turbidity was present, then the absorbance of sample mixture was checked at 660 nm wavelength using UV spectrophotometer. The reading was taken in triplet. Formula used to calculate absorbance is given below.

% inhibition = [(Ac-At)/ Ac] x 100

Where,

Ac = absorbance of control sample.

At = absorbance of test sample.

2.6. Statistical analysis

Statistically all data was expressed as mean ± S.E.M. One way ANOVA followed by Dunnett's multiple comparison test using SPSS 15.0 software was used to evaluate the results. The results were considered to be statistically significant if the P-values were less than 0.05.

3. RESULTS

3.1. Phytochemical analysis

The analysis of phytochemical constituents showed presence of alkaloids, tannins, carbohydrates, phenols and fats in all three extracts of *Pteris vittata* **(Table 1)**.

3.2. Hepatoprotective activity

3.2.1. Effect of different extracts of Pteris vittata on biochemical parameters

Extracts (n-hexane, chloroform and methanol) of *Pteris vittata* were evaluated for their hepatoprotective activity against CCl_4 induced hepatotoxicity. After CCl_4 administration, the normal group of rats showed a significant increase in ALT, AST, total bilirubin and cholesterol levels. Treatment with different extracts of *Pteris vittata* at dose 300 mg/kg showed significant decreased in biochemical parameters i.e. ALT, AST, total bilirubin and cholesterol when compared with CCl_4 group as shown in the **Table 2 and Figure 1**.

3.2.2. Histopathological inspection

Microscopic examination of livers of normal rats showed normal hepatic architecture **(Figure 2a)**. Classic centrilobular necrosis (hepatocyte vacuolation and ballooning, deposition of lipid droplets in hepatocytes and infiltration of inflammatory cells) was observed in liver sections of untreated CCl_4 intoxicated rats **(Figure 2b)**. However, this centrilobular necrosis was markedly inhibited by treatment with silymarin **(Figure 2c)** and by all extracts of *Pteris vittata* **(Figure 2d, 2e and 2f)**. An increase in the degenerative regions, the number of degenerative hepatocytes and the number of inflammatory cells infiltrated in hepatic parenchyma was observed in CCl_4 control group. These changes were ameliorated by treatment with all *Pteris vittata* extracts at 300 mg/kg and by silymarin at 200 mg/kg. The methanol extract of *Pteris vittata* exhibited the strongest effect, followed by the chloroform extract and n-hexane extract.

3.3. Evaluation of in- vitro anti-inflammatory activity

In-vitro anti-inflammatory activity of *Pteris vittata* was performed by inhibition of albumin denaturation method. The chloroform and n-hexane extract of *Pteris vittata* has shown more inhibitory effect as compared to standard drug diclofenac sodium. The IC_{50} of chloroform and n-

hexane extracts were 614µg/L and 661 µg/L respectively, which were less than IC_{50} of diclofenac sodium (704 µg/L), thus showing that chloroform and n- hexane extracts exhibited maximum anti-inflammatory activity. The results of the activity are shown in **Table 3 and Figure 3**.

Table 1: Phytoconstituents in various extracts of *Pteris vittata*

Detection test	n-hexane extract	Chloroform extract	Methanol extract
Alkaloids	+++	+++	++
Glycosides	+++	-	+++
Tannins	+++	++	+++
Proteins	-	-	-
Carbohydrates	+++	+++	+++
Fats	+++	+++	+++
Phenols	+++	+++	+++

"+" represents weak presence, "++" moderate presence, "+++" strong presence and "–" represents absence

Table 2: Assessment of biochemical parameters of different extracts of *Pteris vittata*

Groups	ALT (IU/L)	AST (IU/L)	Total bilirubin (mg/dL)	Cholesterol (mg/dL)
Control	37.67 ± 1.96*	74.4 ± 3.14*	1.404 ± 0.01*	105.00 ± 3.53*
CCl_4	230.00 ± 5.0	255.400 ± 3.00	3.98 ± 0.08	216.00 ± 4.30
Silymarin	54.40 ± 2.7*	90.40 ± 4.57*	1.556 ± 0.07*	131.200 ± 3.56*
n-hexane extract	125.20 ± 3.5*	173.00 ± 4.63*	3.228 ± 0.07*	179.200 ± 1.77*
Chloroform extract	83.40± 2.6*	131.00 ± 3.45*	2.100 ± 0.09*	160.200 ± 2.13*
Methanol extract	69.00± 2.7*	98.60 ± 3.31*	1.6940 ± 0.02*	146.00 ± 2.21*

The values are expressed as mean ± SEM (n=5). Results were found to be statistically significant (*P<0.05) when compared with CCl_4 group

Table 3: *In- vitro* anti-inflammatory activity of different extract of *Pteris vittata*

Samples	Concentrations (µg/L)	Absorbance±S.E.M	%Inhibition	IC_{50} (µg/L)
Control	-	0.820 ±0.000577	-	-
n-hexane extract	25 50 100 250 500 800 1000	0.743 ± 0.015 0.736 ± 0.031 0.646 ± 0.015 0.556 ± 0.005 0.473 ± 0.005 0.356 ± 0.015 0.27 ± 0.01	9.75 10.97 21.95 32.92 42.68 57.31 67.07	661
Chloroform extract	25 50 100 250 500 800 1000	0.673 ± 0.011 0.586 ± 0.005 0.543 ± 0.015 0.48 ± 0.01 0.443 ± 0.011 0.386 ± 0.005 0.283 ± 0.005	18.29 29.26 34.14 41.46 46.34 53.65 65.85	614
Methanol extract	25 50 100 250 500 800 1000	0.756 ± 0.102 0.73 ± 0.001 0.692 ± 0.004 0.582 ± 0.027 0.538 ± 0.004 0.519 ± 0.010 0.376 ± 0.005	7.8 10.97 15.73 29.14 34.26 36.82 54.14	954.69
Diclofenac sodium	25 50 100 250 500 800 1000	0.715 ± 0.001 0.656 ± 0.001 0.615 ± 0.002 0.529 ± 0.001 0.512 ± 0.001 0.33 ± 0.001 0.322 ± 0.001	12.92 20 25 27.8 37.7 59.75 60.73	704

Values are expressed as mean ± S.E.M. (n=3)

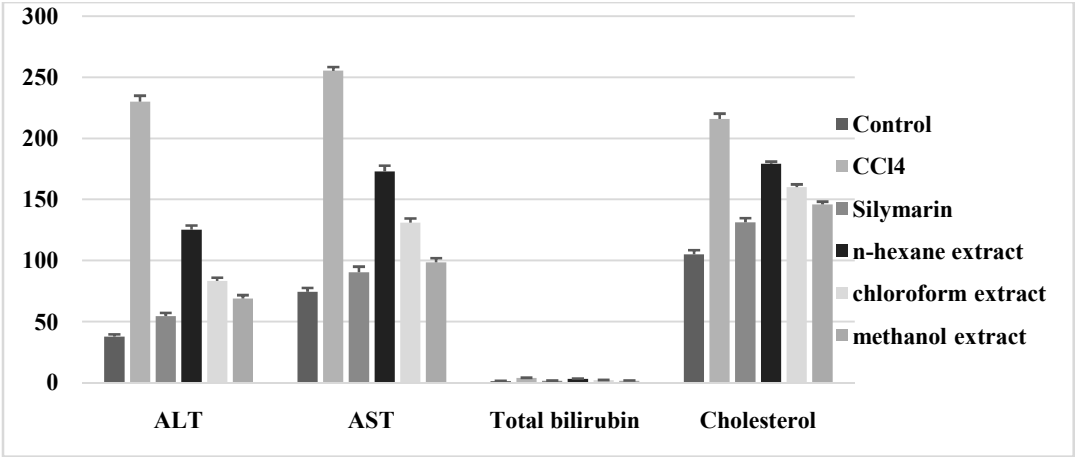

Figure 1: Effect of *Pteris vittata* extracts on biochemical parameters i.e. ALT, AST, bilirubin, cholesterol when compared with CCl_4 group and Silymarin group

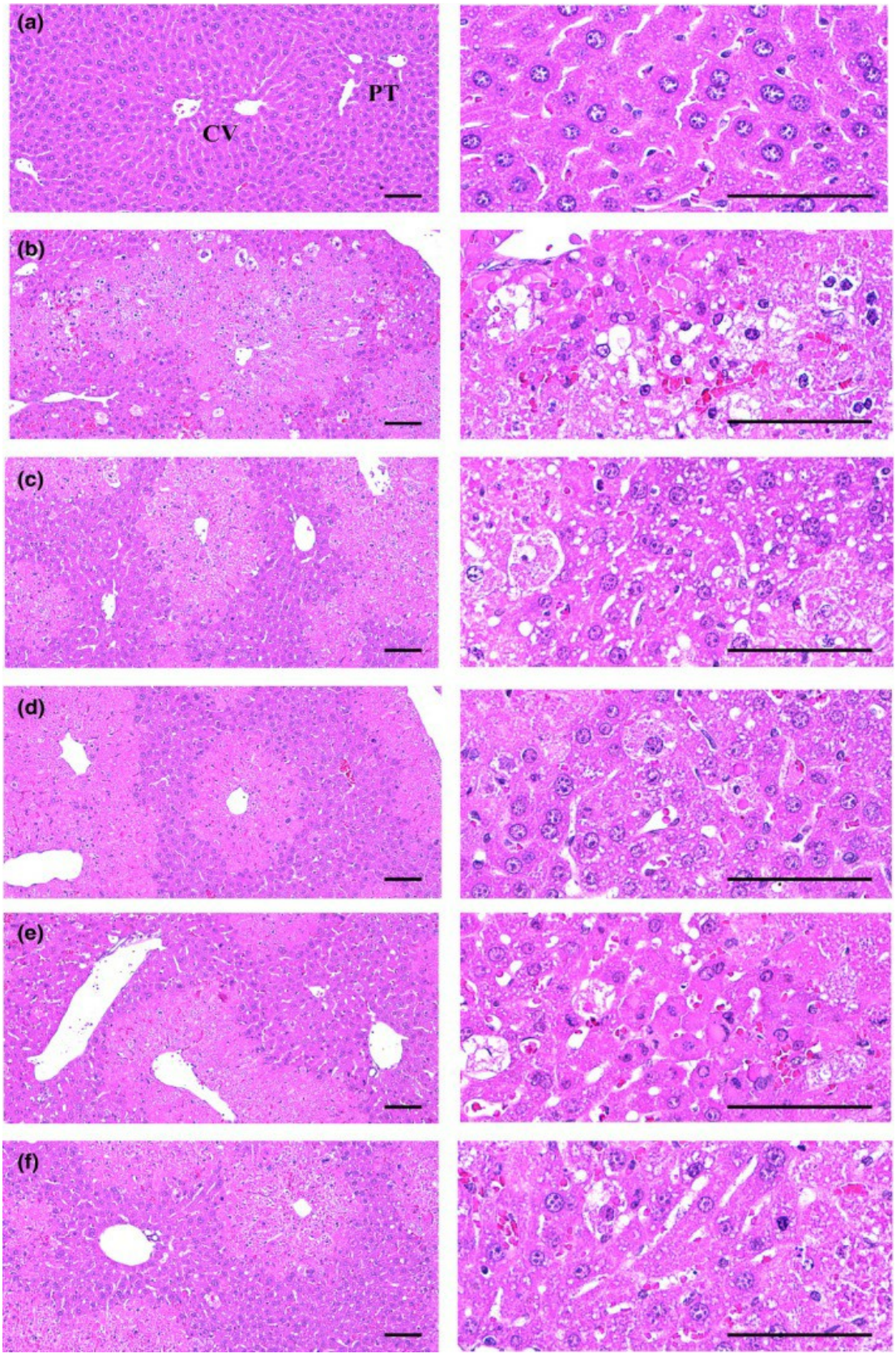

Figure 2: Histopathology of liver sections isolated from each group. (a) Control (b) CCl_4 control (c) Silymarin control (d) n-hexane extract (e) Chloroform extract (f) Methanol extract; (CV: Central vein, PT: Portal triad regions, Hematoxylin-eosin stain, Scale bars = 120 µm)

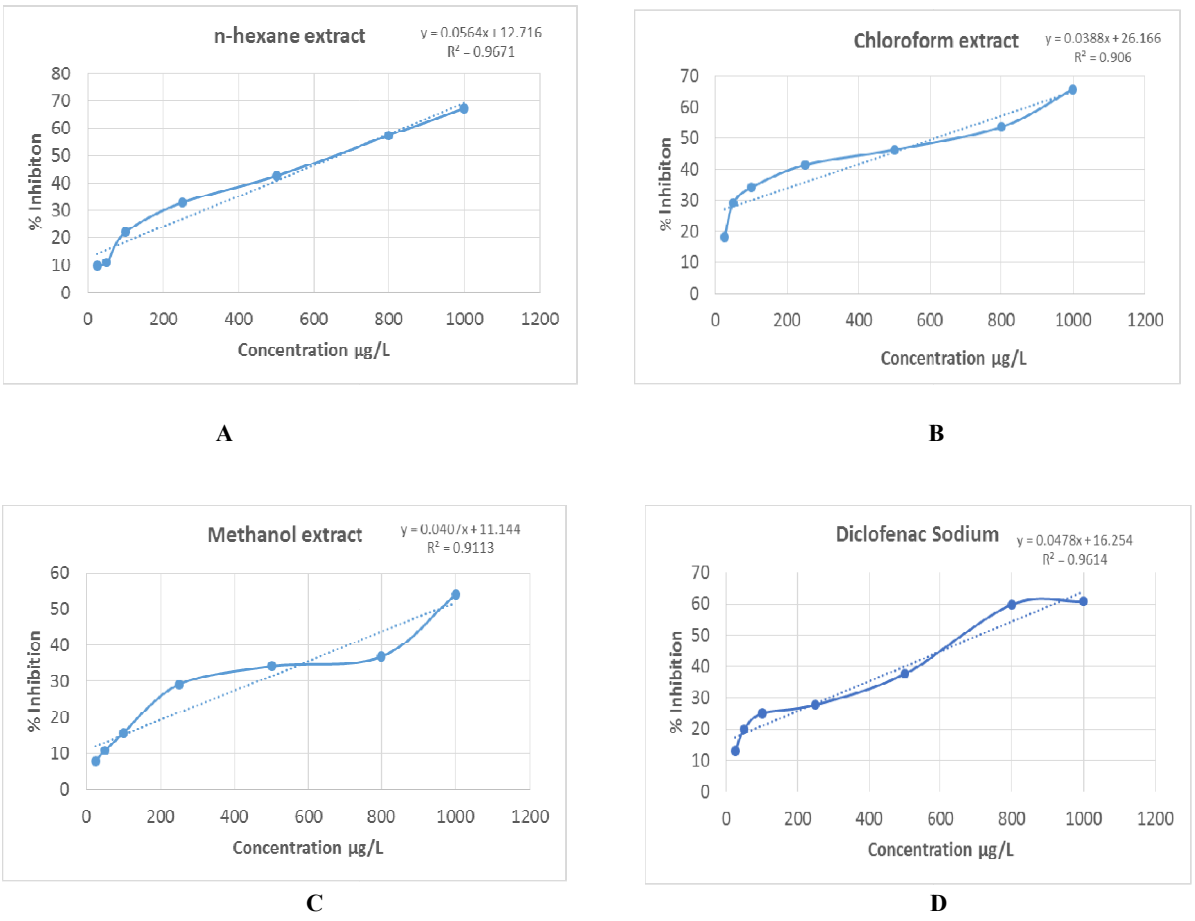

Figure 3: Effect of different *Pteris vittata* extracts on inhibition of protein (egg albumin) denaturation (A) n-hexane extract, (B) chloroform extract, (C) methanol extract and (D)diclofenac sodium at various concentrations

4. DISCUSSION

Phytochemical screening is done for isolating various plant components to assess biotic activity and therapeutic usefulness. In plants therapeutic significance depends on existence of definite chemical constituents having explicit physiologic activity **[18]**. The three extracts of *Pteris vittata* were evaluated for phytochemical screening. The result of phytochemical screening confirmed the presence of different phytoconstituents i.e. alkaloid, tannins, glycosides, carbohydrate, fats, phenols and absence of proteins in all the three extracts. The existence of these highly polar phytoconstituents along with other simple carbohydrates in *Pteris vittata* plant might be the likely cause behind the hepatoprotective activity exhibited by *Pteris vittata.*

The drug induced hepatotoxicity is the major cause of liver damage. Many herbal drugs have been identified to have hepatoprotective activity, for example, *Murraya koenigi, Feronia limonia, Tecomella undulata* etc **[19]**. The three extracts of *Pteris vittata* i.e. n- hexane, chloroform and methanol were evaluated

for their hepatoprotective activity using *in-vivo* animal model i.e. Swiss albino rats against CCl_4 induced hepatotoxicity. CCl_4 is a hepatotoxic agent that becomes activated in the presence of CYP450 enzymes and is converted to its metabolite trichloromethyl (CCl_3^-) free radicals, which can also convert to trichloromethyl peroxy radical ($CCl_3O_2^-$) in the presence of oxygen. Both these metabolites are highly reactive, with CCl_3 free radical binding to biomacromolecules and CCl_3O_2 radical being involved in oxidation of poly unsaturated fatty acids of cellular membranes, thus altering the integrity and permeability of cell membrane and causing leakage of liver enzymes into blood stream **[20]**. The effect of all the three extracts of *Pteris vittata* at a dose of 300 mg/kg on the biochemical parameters i.e. ALT, AST, total bilirubin and cholesterol were determined. Leakage of cellular enzymes ALT and AST into plasma occurs due to disturbances caused in transport functions of hepatocytes thereby indicating sign of hepatic injury. Hepatic injury is better investigated by ALT values, as it is more specific to the normal liver functioning. Increased AST levels indicate the cellular leakage and loss of functional ability of cell membrane in liver. Bilirubin originates from breakdown of heme in the reticuloendothelia system. It gets converted to the conjugated form by entering microsomes of hepatocytes. In bile it is present in this conjugated form and is passed along bile ducts into the intestine. High levels of bilirubin in blood indicate reduced conjugation or decreased bilirubin transport. Bilirubin levels are used as an index to evaluate normal liver functioning instead of hepatocellular injury **[21]**. Cholesterol is a precursor in the synthesis of bile acids. Biliary secretions contribute to the presence of cholesterol in the gut. High levels of cholesterol, known as hypercholesterolemia, can occur in obstructive jaundice, hemolytic jaundice and liver damage **[22]**. Silymarin was used as standard drug. Its source are the seeds of *Silybum marianum* which is an edible plant being employed from primitive times for herbal treatment of various liver diseases **[23]**. It was observed that serum level of biochemical parameters of the group treated with CCl_4 were increased, while the groups treated with plant extracts showed decrease in serum level of biochemical parameters. Significant decrease in biochemical parameters was also observed in a group treated with standard drug i.e. silymarin at a dose of 200 mg/kg. The biochemical parameters of all the six groups i.e. control group, CCl_4 treated group, n-hexane group, and chloroform group, methanol group and silymarin

treated group were studied. Methanol extract of *Pteris vittata* had shown significant decrease in biochemical parameters as compared to n-hexane and chloroform extracts when compared to standard drug. In correlation to the biochemical parameters, the degenerative regions, the number of degenerative hepatocytes and the number of inflammatory cells infiltrated in hepatic parenchyma were high in the CCl_4 control compared with the normal control. However, the groups treated with extracts of *Pteris vittata* showed significant inhibition in the CCl_4 treatment-related centrilobular hepatic damage. Thus, the histopathological findings provide evidence that all *Pteris vittata* extracts in this study exert hepatoprotective effects, with the methanol extract in particular showing most favorable hepatoprotective effect. Thus, it has been proved that methanol extract of *Pteris vittata* has hepatoprotective potential and can be used as hepatoprotective agent.

In the present study, the protein denaturation bioassay was studied to evaluate *in-vitro* anti-inflammatory properties of three extracts (n-hexane, chloroform and methanol) of *Pteris vittata*. Denaturation is a process in which the protein loses its secondary and tertiary structure by stress or any chemical agent. The major cause of inflammation is protein denaturation. Previously, the effect of different plant extracts on protein denaturation have been evaluated, for example, *Camellia sinensis, Albuca setosa etc* **[17]**. In the present study IC_{50} (half maximal inhibitory concentration) and percentage inhibition of extracts were determined to evaluate *in-vitro* anti-inflammatory activity. IC_{50} gives information about how much a drug or a compound is used to inhibit the specific biological function. Lower value of IC_{50} means more ability of a drug or compound and more pharmacological activity. IC_{50} of chloroform and n-hexane extracts was less than IC_{50} value of diclofenac sodium, whereas IC_{50} value of the methanol extract was higher than the IC_{50} value of diclofenac sodium. Thus, the present study confirmed that chloroform and n-hexane extract of *Pteris vittata* has maximum anti-inflammatory activity at different concentrations on protein denaturation when compared to diclofenac sodium.

Phenolic compounds are present in plants and are well known to possess several notable biological properties **[24]**. Phenolic compounds are sub classified into phenolic acid, flavonoid and tannin. Caffeic acid, chlorogenic acid, rutin, quercetin, apigenin, kaempferol and luteolin are some of the phenolic compounds that have been reported to be present in *Pteris vittata* **[11]**. Number of hydroxyl groups on the

aromatic ring in phenolic compounds is responsible for radical scavenging activity **[25]**. The phenolic compounds delay or inhibit progression of various diseases by preventing peroxidation of lipid membrane **[26-27]**. Activated neutrophils and macrophages release reactive oxygen species (ROS) causing inflammation injury and this ROS induced inflammation in turn stimulates release of cytokines (interleukin-1, tumor necrosis factor-α and interferon-α). But, the main mediators promoting the inflammatory process are the free radicals and thus, the antioxidants by neutralizing these free radicals reduce inflammation **[11]**. Thus, in the present study, the hepatoprotective and anti-inflammatory activity can be attributed to the presence of polyphenols, particularly anti-oxidative flavonoids.

5. CONCLUSION

Pteris vittata was not investigated previously for *in-vivo* hepatoprotective and *in-vitro* anti-inflammatory activity. The present study showed that *Pteris vittata* has both hepatoprotective and anti-inflammatory potentials. This plant can be used in future in the formulation of medicines for the treatment of different liver conditions and inflammation.

ACKNOWLEDGMENTS

We are very thankful to the management of The University of the Lahore for allowing us to utilize their laboratories and instruments for this research work.

CONFLICT OF INTEREST

Author found no conflict of interest.

REFERENCES

[1] Nemeth E, Baird AW, O'Farrelly C. Microanatomy of the liver immune system. Seminars in Immunopathology, 2009, 31(3) 333-343.

[2] Dienes HP, Drebber U. Pathology of immune-mediated liver injury. Digestive Diseases. 2010, 28(1) 57-62.

[3] Carithers RL Jr., Macclain CJ. Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology, Diagnosis, Management (Feldman M, Friedman LS and Brandt LJ, eds). 9th ed, Saunders/Elsevier Publishers, Philadelphia, USA, 2010, 1383-1400.

[4] Joshy C, Thahimon PA, Kumar RA, Carla B, Sunil C. Hepatoprotective, anti-inflammatory and antioxidant activities of Flacourtia montana J. Grah leaf extract in male Wistar rats. Bulletin of Faculty of Pharmacy, Cairo University, 2016, 54(2) 209-217.

[5] Akula US, Odhav B. In vitro 5-lipoxygenase inhibition of polyphenolic antioxidants from

undomesticated plants of South Africa. Journal of Medicinal Plants Research, 2008, 2(9) 207-212.

[6] Sarkhel S. Evaluation of the anti-inflammatory activities of Quillaja saponaria Mol. saponin extract in mice. Toxicology reports, 2016, 3, 1-3.

[7] Owolabi OJ, Omogbai EK, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. African Journal of Biotechnology, 2007, 6(14) 1677-1680.

[8] Bondada B, Tu C, Ma L. Surface structure and anatomical aspects of Chinese brake fern (Pteris vittata; Pteridaceae). Brittonia, 2006, 58(3) 217-228.

[9] Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED. A fern that hyperaccumulates arsenic. Nature, 2001, 409(6820) 579.

[10] Cao H, Chai TT, Wang X, Morais-Braga MF, Yang JH, Wong FC, Wang R, Yao H, Cao J, Cornara L, Burlando B. Phytochemicals from fern species: potential for medicine applications. Phytochemistry reviews, 2017, 16(3) 379-440.

[11] Baskaran XR, Vigila AV, Zhang SZ, Feng SX, Liao WB. A review of the use of pteridophytes for treating human ailments. Journal of Zhejiang University-SCIENCE B, 2018, 19(2) 85-119.

[12] Chai TT, Yeoh LY, Ismail NM, Ong HC, Manan FA, Wong FC. Evaluation of glucosidase inhibitory and cytotoxic potential of five selected edible and medicinal ferns. Tropical Journal of Pharmaceutical Research, 2015, 14(3) 449-54.

[13] Tirkey N, Pilkhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, and decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. BMC pharmacology, 2005, 5(1) 2.

[14] Hussain F, Malik A, Ayyaz U, Shafique H, Rana Z, Hussain Z. Efficient hepatoprotective activity of cranberry extract against CCl4-induced hepatotoxicity in Wistar albino rat model: Down-regulation of liver enzymes and strong antioxidant activity. Asian Pacific journal of tropical medicine, 2017, 10(11)1054-8.

[15] Sobeh M, Mahmoud MF, Abdelfattah MA, El-Beshbishy HA, El-Shazly AM, Wink M. Hepatoprotective and hypoglycemic effects of a tannin

rich extract from Ximenia americana var. caffra root. Phytomedicine, 2017, 33, 36-42.

[16] Alharbi KB, Mousa HM, Ibrahim ZH, El-Ashmawy IM. Hepatoprotective effect of methanolic extracts of Prosopis farcta and Lycium shawii against carbon tetrachloride-induced hepatotoxicity in rats. Journal of Biological Sciences, 2017, 17, 35-41.

[17] Chatterjee P, Chandra S, Dey P, Bhattacharya S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative in vitro study. Journal of advanced pharmaceutical technology & research, 2012, 3(2), 136.

[18] Tatiya A, Surana S, Bhavsar S, Patil D, Patil Y. Pharmacognostic and preliminary phytochemical investigation of Eulophia herbacea Lindl. Tubers (Orchidaceae). Asian Pacific Journal of Tropical Disease, 2012, 2, S50-5.

[19] Desai SN, Patel DK, Devkar RV, Patel PV, Ramachandran AV. Hepatoprotective potential of polyphenol rich extract of Murraya koenigii L.: an in vivo study. Food and chemical toxicology, 2012, 50(2) 310-4.

[20] Islam M, Hussain K, Latif A, Hashmi FK, Saeed H, Bukhari NI, Hassan SS, Danish MZ, Ahmad B. Evaluation of extracts of seeds of Syzygium cumini L. for hepatoprotective activity using CCl4-induced stressed rats. Pakistan Veterinary Journal, 2015, 35(2), 197-200.

[21] Abirami A, Nagarani G, Siddhuraju P. Hepatoprotective effect of leaf extracts from Citrus hystrix and C. maxima against paracetamol induced liver injury in rats. Food science and human wellness, 2015, 4(1), 35-41.

[22] Kumar V, Gill KD. Basic concepts in clinical biochemistry: A practical guide. 1st Ed, Springer Nature, Singapore, 2018, 93-95.

[23] Govind P, Sahni YP. A review on hepatoprotective activity of silymarin. International Journal of Research in Ayurveda and Pharmacy, 2011, 2(1) 75-9.

[24] Bhattacharya S. Are we in the polyphenols era? Pharmacognosy Res, 2011, 3(2) 147-147.

[25] Lazarova I, Zengin G, Aktumsek A, Gevrenova R, Ceylan R, Uysal S. HPLC–DAD analysis of phenolic compounds and antioxidant properties of Asphodeline lutea roots from

Bulgaria and Turkey. Industrial crops and products, 2014, 61, 438-41.

[26] Kumar S, Gupta A, Pandey AK. Calotropis procera root extract has the capability to combat free radical mediated damage. ISRN pharmacology, 2013.

[27] Kumar S, Chashoo G, Saxena AK, Pandey AK. Parthenium hysterophorus: a probable source of anticancer, antioxidant and anti-HIV agents. BioMed Research International, 2013.