In vitro investigation of phytochemicals, Antioxidant and Hepatoprotective activity of *Selaginella bryopteris*

Deena Prakash[1]; Divya Bisht[1], Deepa Yadav[2]; Arvind K. Shakya[1]; Sadhana Shrivastava[2]; Sangeeta Shukla[2] and Ramesh Kumar[3.] [1]Biochemistry Discipline, School of Sciences, Indira Gandhi National Open University, New Delhi 110068, India. [2]School of Studies in Zoology, Jiwaji University, Gwalior. [3]Department of Biochemistry, Bundelkhand University, Jhansi- 284 128 (U.P.)

Abstract:

The present study was aimed to evaluate phytoconstituents, total phenolic content, free radical scavenging activity and *in vitro* hepatoprotective effect of *Selaginella bryopteris* (SB) against acetaminophen (APAP) and carbon tetrachloride (CCl_4) induced hepatotoxicity on HepG2 cell lines. The preliminary phytochemicals screening of aqueous and ethanolic extract of SB confirmed the presence of phytochemical constituents and total phenolic contents. Both SB extracts exhibited free radical scavenging activities in dose dependent manner (100-500 µg/mL). Ethanolic extract at higher concentration (500 µg/mL) showed highest DPPH, ABTS and H_2O_2 free radical scavenging activity followed by the SB aqueous extract. *In vitro* hepatoprotective activity was assessed by MTT assay confirmed the dose-dependent cellular protection (72%-88%) by SB extracts at three doses (25µg/mL, 50µg/mL and 100µg/mL) against CCl_4 and APAP induced toxicity in HepG2 cell line, the cell viability rate was found to be higher at 100µg/mL in SB ethanolic extract compared to the SB aqueous extract. No adverse effect was observed in the SB extract alone treated HepG2 cell line. The results indicated that *Selaginella bryopteris* extract has significant *in vitro* hepatoprotective activity against APAP and CCl_4-inducedtoxicity which might be due to the presence of free radical scavenging phytoconstituents.

Keywords: Antioxidant, APAP, CCl_4, Hepatoprotection, HepG2 cell line, Polyphenols, *Selaginella bryopteris.*

*Address for Correspondence: arvind.kumar@ignou.ac.in

Introduction:

Liver is the main metabolic and drug detoxification organ of the human body. It plays an important role in metabolism, glucose storage, bile acid synthesis, and maintaining the body's homoeostasis (Mamri et al. 2022). It plays a crucial role in the detoxification of endogenous and exogenous xenobiotics. The liver CYP450 system metabolizes the majority of drugs and chemicals. Biological agents (pathogenic bacteria and viruses) as well as chronic dose of chemicals, drugs, alcohol, acetaminophen, and synthetic drugs contribute to cause liver damage (Tripathi, 2013). The prevalence of liver diseases such as cirrhosis, jaundice, viral hepatitis and hepatic carcinoma has increased worldwide in recent years and are responsible for approximately 2 million deaths annually (WHO, 2019). According to (Wang et al. 2017) and (Jannu 2012), oxidative stress induced hepatotoxicity results from an imbalance between the antioxidant defence system and oxidative stress. In India, liver diseases are the 10th most common cause of mortality. It is an emerging global health issue and is reported to be higher in India (8-30%) (Panda et al. 2015). In spite of tremendous scientific advancement in the field of medical sciences in recent years, liver related health problems are rising in modern society.

Medicinal plants are considered to be a significant source of therapeutic medicine in the Indian traditional medical system (Jain et al. 2015; Imrana and Asif 2020). Scientific communities have reported that herbal plants and their products have various medicinal properties for the treatment of human diseases including hepatic diseases. In this regard, plants with traditional therapeutic values are being investigated primarily for their scientific validation by *in vivo* and *in vitro* studies (Singh and Singh 2015). *Selaginella bryopteris* (Linn.) is a lithophytic pteridophyte herb belonging to the family Selaginellaceae. It is found in Uttarakhand, Uttar Pradesh, Orissa, Biharand the Aravalli Mountains of India. This herb is locally known as Sanjeevani (a plant that offers life). Due to its ability to grow and survive in extreme drought conditions, it has remarkable resurrection abilities (Sah, 2008). However, *Selaginella bryopteris* has been used by tribal people for the treatment of common health problems such as jaundice, dysentery, skin inflammation, urinary infection, fever and gynaecological problems (Sah 2008; Ganeshaiah 2009; Antony and Thomas

2011). It has been reported to have medicinal properties like anticancer (Mishra et al. 2011), anti-inflammatory (Paswan et al. 2017), antimicrobial (Singh et.al. 2018), antioxidative stress (Pandey et al. 1999; Sah et al. 2005) and antiprotozoal activity (Kunert et al. 2008). The SB plant has been reported to have phytochemicals like amentoflavone and hinokoflavone which possess antiprotozoal and antimicrobial activities (Verma et al. 2015). This plant is also a rich source of flavonoids, tannins, saponins alkaloids and phenols (Setyawan and Darusman 2008). However, this plant has not been scientifically studied for its hepatoprotective properties.

Therefore, the present study was undertaken to investigate *Selaginella bryopteris* for its antioxidant and hepatoprotective potential against carbon tetrachloride (CCl_4) and acetaminophen-induced oxidative damage in HepG2 cell line.

Materials and methods:

Drugs and Chemicals

Human liver hepatoma cell line (HepG2) was procured from the National Centre for Cell Science (NCCS), Pune, India. Silymarin was from Sigma Aldrich (USA), acetaminophen and carbon tetrachloride were procured from E-Merck (Germany). 1,1-diphenyl-2-picrylhydrazine (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Hydrogen peroxide (H_2O_2), Ascorbic acid and Gallic acid were purchased from Himedia. India. All chemicals used were of analytical grade.

Collection of plant sample

The *Selaginella bryopteris* was obtained in the month of December-January, 2018 at the forest area of Aurangabad district, Bihar, India (Fig.1a). The plant material was also commercially available at Khari Baoli, Delhi, India and is used as an ornamental herb in homes. Authentication of the plant species (Fig. 1b) was done by Department of Botany, Delhi University (DU), New Delhi and the specimen of the plant was deposited in the herbarium of the Botany Department, DU (Voucher number-DUH14498).

Fig. 1a: *Selaginella bryopteris herb*

Fig.1b. *Selaginella bryopteris* (dried)

Preparation of Plant extract

The whole part of *Selaginella bryopteris* (SB) was thoroughly washed with distilled water and air dried at room temperature (25-30°C) for 10-15 days. To prepare aqueous extract and ethanolic extract, SB was grinded into fine powder and subjected as followed-

Preparation of aqueous extract: The dried powder of SB was mixed with distilled water (100/400 w/v) and heated for about 4 hours at 40-50°C. After cooling, the Aqueous mixture was subjected to filtration by Whatman No.1 filter paper and extracted by evaporation of water in regulated water bath at 50-60°C (Al-Manhel and Niamah 2015). The percentage yield of the SBA was found to be 20% (w/w). The dried extract of *Selaginella bryopteris* (SBA) was stored in sterile tube at 4°C.

Preparation of Ethanolic extract: The dried powder of SB was mixed in ethanol (100/500 w/v) and subjected to shaking for 5 days (2 hours/day) and the extract obtained was filtered using Whatman No. 1 filter paper and evaporated at 50-60°C in a regulated water bath. This step is repeated to prepare an adequate amount of ethanolic extract (Owoyele et al. 2008). The percentage yield of *Selaginella bryopteris* ethanolic extract (SBE) was found to be 3.5% (w/w). The resulting ethanolic extract (SBE) was stored at 4 ºC.

Detection of Phytochemical constituents: The phytochemicals of both SB extract were determined as per the methodologies of Harborne (Harborne 1998) as followed:

Flavonoids: 1mL of 1N NaOH was added to 2 mL of plant extract, as a result yellow colour was formed which was turn into colourless after addition of 1 ml of diluted HCl. Colourless solution confirmed the presence of flavonoids.

Reducing sugar: 1 mL of plant extract was taken in which equal amount of Fehling A and B reagent were added and heated at 60°C. Appearance of brick red colour confirmed the presence of reducing sugar.

Tannins: 1mL $FeCl_3$ solution was added into the 1 ml of plant extract where green black colour was formed that indicating the presence of tannins.
Coumarins: The addition of 2 ml of plant extract and 3mL of 10% NAOH produced yellow colour which confirms the presence of coumarins.

Anthocyanins:2mL of plant extract and 2 mL of dil. H_2SO_4 and ammonia were mixed and then the pink red coloration was appeared in sample test tube which suggested the presence of anthocyanins.

Saponins: The diluted extract was dissolved in 10 ml of distilled water in the test tube and vortex for 15 minutes. The formation of 1cm layer of foam indicating the presence of saponins.

Steroids:1 mL of plant extract was dissolved in chloroform and then 1 mL of Conc. H_2SO_4 was added slowly where chloroform acquired reddish blue colour and acid layer green fluorescence which showed the presence of steroids.

Alkaloids: Few drops of diluted HCl and 2 ml of Wagner's reagent were added in 1ml plant extract. The reddish-brown precipitate was formed which exhibits the presence of alkaloids.

Glycosides: 2mL of plant extract was mixed with 2 drops of alcoholic α–naphthol and then in that solution, 1mL conc. H_2SO_4 (along the sides of test tube) was added. The formation of a violet ring depicts the presence of glycosides in plant extract.

Determination of total phenolic content: The total phenolic content in SB extracts (SBA and SBE) was determined spectrophotometrically according to the Folin-Circlet method (Lowry 1951). To 0.5mL sample (in triplicates), 2.5 mL of 10% Folin reagent was added followed by 2.5 ml of 7.5% sodium carbonate (Na_2CO_3). After 2 hours incubation, the absorbance of the sample was measured with a UV-Visible spectrophotometer at 765 nm. Total phenolic content was calculated using a standard curve of concentration versus absorbance. The results were expressed in µg gallic acid/mg of plant material.

Determination of in vitro antioxidant activities: DPPH radical, ABTS radical, and H_2O_2 radical assays were used for the determination of the antioxidant activity of both extracts (SBA and SBE) at different concentrations i.e., 100-500 µg/mL. Each test was done in triplicate. The antioxidant ability of SB extract was expressed as IC50 values (Inhibitory concentrations of samples required

to neutralize 50% of free radicals). IC50 values were calculated by linear regression analysis. Free-radical scavenging activity was expressed as percent inhibition (%).

The percentage inhibition (%) of free radical scavenging activity (DPPH, ABTS and H_2O_2) of plant extract was calculated using the following formula.

Percent inhibition (%) = [(Ao − A1/Ao) x 100]

Where Ao is the absorbance of the control and A1 is the absorbance in test sample.

DPPH assay: DPPH (1, 1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity of plant extract was measured (Shimada et al.1992). The reaction mixture contains 3ml of SBA and SBE extract solution (100-500 µg/ml) and 1ml of solution 0.1mM DPPH in ethanol. The mixture was subjected to vortex thoroughly and kept in dark at room temperature for 30 minutes. After 30 minutes incubation, the absorbance of test sample was measured at 517nm by UV-Visible spectrophotometer. Ascorbic acid was used as a positive standard (10-50 µg/ml).

ABTS assay*:* The ABTS•+ scavenging activity of plant extract was measured by the method of (Re et al.1999). The 7 mM ABTS solution prepared in ethanol was mixed with 2.45 mM potassium persulphate and then it was allowed to stand in the dark at room temperature for 14-16 h before use. ABTS•+ solution was diluted with the methanol and its 0.9 ml was added to the test tube containing 0.1 ml of plant extract (SBE and SBA) at the dose concentrations (100-500 µg/ml). The mixture incubated for 15 minutes and then absorbance was measured spectrophotometrically at 734 nm. Ascorbic acid was used as standard (10-50 µg/ml).

Hydrogen peroxide (H_2O_2) assay: The hydrogen peroxide scavenging activity of plant extract was determined by method of Ruchet al.1989. 3.4 ml of 0.1 M phosphate buffer (pH 7.4) was mixed with 1ml of plant extract (SBA and SBE) at the different concentration (100-500 µg/ml) and then 0.6 ml of 43mM hydrogen peroxide was added to it. After 10 min. incubation of reaction mixture, the absorbance at 260 nm was recorded. For each concentration, mixture without sample was used

as control and mixture without H_2O_2 was used as blank. Ascorbic acid was used as standard (10-50 µg/ml).

Assessment of in vitro hepatoprotective activity of SB extract

Cell Culture: HepG2 (Human Hepatoma cell lines) were cultured (1×105 cells) with Dulbecco's modified Eagle medium ((DMEM) added with 10% Fetal Bovine serum (FBS) and antibiotics like penicillin (100U/mL) and streptomycin (100 µg/mL) in a humidified atmosphere of 5% Carbon dioxide at 37ºC until confluent (Pareek et.al., 2013). The MTT assay was performed in triplicates (n=3) to assess the cell viability of treated HepG2 cell lines (Mossmann 1983). The three concentrations at 25 µg/ml, 50 µg/ml and 100 µg/ml) were used for SB extracts (SBA and SBE) and positive control, silymarin (5 mg /ml stock solution) for the treatment of cells.

In vitro cytotoxic effect of SB extract: The cytotoxic effect of aqueous and ethanolic extract of SB was assessed by MTT assay [3- (4,5 dimethyl thiazol-2yl) -2,5 diphenyl tetrazolium bromide)]. HepG2 cells (1×10^5 cells/ml/well) were seeded in 96-well culture plate for 24 h in CO_2incubator. The SB extract at different concentrations (25 µg/ml, 50 µg/ml and 100 µg/ml) and the positive control silymarin were added in the cell culture medium (Elisa plate well) after 24 h incubation period. After next 24 hours of incubation, 10 µL of MTT reagent was added in each well. The plates were kept on shaker for 10 minutes followed by the incubation for 4 h at 37 ºC. After 4 h, the supernatant was removed and 200 µL of DMSO was added and the plates were gently shaken. The absorbance (OD) was measured at 570 nm by an ELISA plate reader.

The percentage of cell viability was calculated by the following formula:

Cell Viability (%): Mean OD of Treated group/ Mean OD of Control group) ×100

Effect of SB extract on CCl_4-induced toxicity: HepG2 cells (1×105 cells/well) were seeded in 96 well culture plate and incubated for 24 hours in CO_2 incubator. All the groups (1-11) were treated as follows subjected to 24 hours incubation. The CCl_4 at 1% concentration (Jena et al. 2019) was added along with plant extract and silymarin as a conjoint treatment to the cell culture 96 Elisa

well plates. Group 1 treated with DMEM Medium (Normal control). Group 2 cells treated with 1% (v/v) CCl_4; Groups 3-5 (as in Group 2) treated with SBA (25 µg/ml, 50 µg/ml and 100 µg/ml) along with 1% CCl_4. Groups 6-8 (as in Group 2) treated with SBE at 25 µg/ml, 50 µg/ml and 100 µg/ml. Groups 9-11 (as in Group 2) treated with silymarin at concentration 25 µg/ml, 50µg/ml and 100 µg/ml. After 24 incubation, 10 µL MTT dye was added into each well and subjected to as per MTT assay protocol. The colour product was measured spectrophotometrically at 570 nm wavelength by Elisa reader.

Effect of SB extract on APAP-induced toxicity: HepG2 cells lines were seeded and treated in a similar manner toCCl_4 treatment. After 24 hours of cell seeding, following groups were made and treatment was given accordingly like in Group 1, DMEM medium was added, in Group 2, 20mM APAP was added (Jannuzzi et. al. 2018), in groups 3-5, cells were given 20mM APAP with 25 µg/ml, 50 µg/ml and 100 µg/ml of SBA. In groups 6-8, treatment of 20mM APAP along with SBE at 25 µg/ml, 50 µg/ml and 100 µg/ml. In the last silymarin groups 9-11, 20mM APAP and 25 µg/ml, 50 µg/ml and 100 µg/ml of silymarin was treated to cells followed by 24 hours of incubation. Finally, MTT dye was added to each well and plate was incubated for 3-4 hours in dark. After incubation, media was discarded and 100 µL DMSO was added to each well and then kept on shaker for 10 minutes. Cells convert the MTT dye into an insoluble purple formazan complex which was measured at 570 nm spectrophotometrically using the ELISA reader.

Statistical analysis: Results were presented as mean ± S.E.M of triplicate used in each group. Data was statistically analysed by ANOVA via In Stat-Graph Pad software, (an open-source statistical software). $P < 0.05$ was considered to be significant.

Results:

Phytochemical analysis: Table 1 showed the presence and absence of the phytoconstituents in aqueous and ethanolic extract of SB. The flavonoids, reducing sugar, anthocyanin, coumarin, steroid and alkaloid were present in both SB extracts. Tannin and saponin were absent in ethanolic extract while glycosides were absent in an SB aqueous extract. The total phenolic content was

found to be 70 µg/mg in aqueous extract and 591 µg/mg in ethanolic extract of SB which is equivalent to as µg gallic acid/mg of plant material (Table 2).

Table 1: Qualitative phytochemicals analysis of *S. bryopteris* (SB) extracts.

Test performed	**Result**	
	Aqueous extract (SBA)	Ethanolic extract (SBE)
Flavonoid	Present	Present
Reducing sugar	Present	Present
Tannins	Present	Absent
Coumarins	Present	Present
Anthocyanins	Present	Present
Saponins	Present	Absent
Steroids	Present	Present
Alkaloids	Present	Present
Glycosides	Absent	Present

Table 2: Total phenolic content of the *S. bryopteris* extract.

SB Extracts	**Phenolic content (µg gallic acid/mg of plant material)**
Aqueous extract (SBA)	70µg/mg
Ethanolic extract (SBE)	591 µg/mg

Antioxidant activity of SB extract: DPPH (2,2-diphenyl-1-picrylhydrazyl) is a free radical blue dye used to determine the free radical scavenging activity of plant extracts. Table 3 showed DPPH free radical scavenging activity of *Selaginella bryopteris* aqueous extract (SBA) and ethanolic extract (SBE) in different concentration (100-500 µg/mL). Both plant extracts showed

DPPH free radical inhibition in dose-dependent manner. The maximum DPPH inhibition was found to be 34% and 72.60% at 500 µg/mL of the SBA and SBE respectively. The IC50 value was found to be 632µg/ml and 263 µg/ml in SBA and SBE respectively.

Table 3: DPPH scavenging activity of *S. bryopteris* extract.

Concentration (µg/mL)	**DPPH Percent inhibition (%)**		
	SBA	SBE	Ascorbic Acid (10-50 µg/mL)
100	17.80 ± 4.52	64.94 ±3.33	66.23 ± 0.22
200	25.06 ± 2.97	67.20 ± 0.66	69.68 ± 0.51
300	26.46 ± 1.40	69.96 ± 0.04	72.13 ± 1.00
400	29.52 ± 0.95	71.60 ± 0.66	74.46 ± 0.49
500	34 ± 0.96	72.60 ± 1.46	79.80 ± 0.46
IC_{50}	632	263	24

Values are mean ± S.E.M., n=3 in each group of triplicate determinations.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) is another assay to determine the free radical scavenging activity of plant extract. ABTS is a cation produced by the reaction of potassium persulfate and reduced in the presence of phytochemicals. Table 4 showed the concentration dependent ABTS radical scavenging activity of SBA and SBE. The maximum percent ABTS inhibition (%) was observed 35.38% and 88.50% at 500 µg/mL of SBA and SBE respectively. The result was compared to ascorbic acid. The IC50 value of ABTS inhibition was found to be 649µg/ml and 232 µg/ml in SBA and SBE respectively.

Table 4: ABTS scavenging activity of *S. bryopteris* extract.

Concentration (µg/mL)	**ABTS percent inhibition (%)**		
	SBA	SBE	Ascorbic Acid (10-50 µg/mL)
100	14.38 ± 1.81	52.80 ± 0.95	53.44 ± 5.52
200	20.22 ± 1.13	69.10 ± 0.51	79.55 ± 0.52
300	25.00 ± 2.10	77.05 ± 1.21	86.70 ± 0.68
400	30.35 ± 1.92	80.25 ± 0.55	97.60 ± 1.11
500	35.38 ± 1.51	88.50 ± 0.98	98.96 ± 1.03
IC_{50}	649	232	20.26

Values are mean ± S.E.M., n=3 in each group of triplicate determinations.

Table 5 exhibits the H_2O_2 scavenging activity of SBA and SBE in concentration-dependent manner. Maximum percentage inhibition of H_2O_2 by the SBA and SBE was found to be 63.67% and 83.25% respectively at500 µg/mL when compared with ascorbic acid. The IC50 value of H_2O_2 inhibition was found to be 326µg/ml and 230 µg/ml in SBA and SBE respectively.

Table 5: Hydrogen peroxide scavenging activity of *S. bryopteris* extract.

Concentration(µg/mL)	**H_2O_2 percent inhibition (%)**		
	SBA	SBE	Ascorbic Acid (10-50 µg/mL)
100	44.71 ± 2.57	70.25 ± 0.91	35.72 ± 0.54
200	47.88 ± 4.31	77.38 ± 0.90	51.82 ± 1.97
300	51.77 ± 4.09	77.88 ± 0.40	57.07 ± 0.82
400	57.91 ± 2.87	80.80 ± 0.56	68.40 ± 0.98
500	63.67 ± 3.20	83.25 ± 0.41	71.03 ± 1.20
IC_{50}	326	230	29.27

Values are mean±S.E.M.,n=3 in each group of triplicate determinations.

Assessment of in vitro hepatoprotective activity of SB extract:

Cytotoxic effect of SB extract: The cytotoxicity of plant extract (SBA and SBE) was investigated using the HepG2 cell line by the MTT assay. Fig. 2 depicts the cytotoxicity effect of aqueous extract *Selaginella bryopteris* (SBA) and ethanolic extract (SBE) at three concentrations (25, 50, 100 µg/mL respectively) on HepG2 cell lines. In this result, plant extract (SBA and SBE) was found to be non-toxic at all three concentrations compared with the control group. Cell viability was found to be about 98% of plant extract which was similar to the control group.

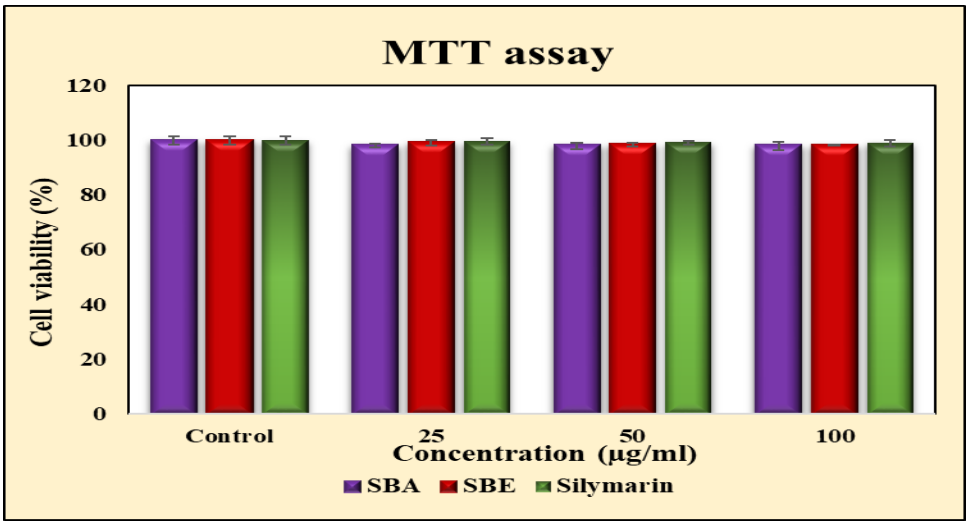
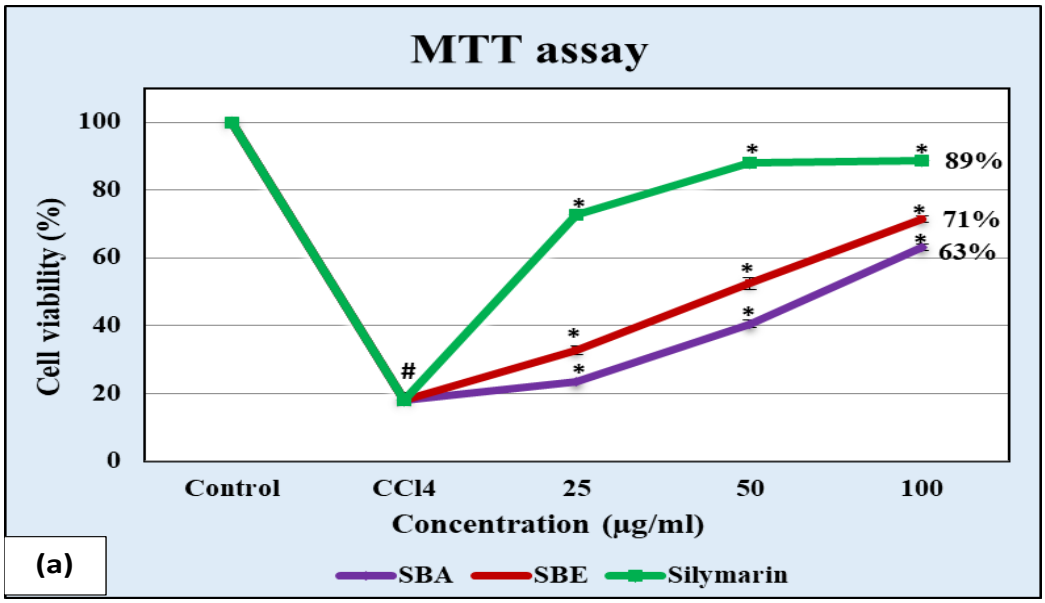


Fig. 2: Cytotoxic effect of SBA and SBE on HepG2 cell line.

Cytotoxic effects of the *Selaginella bryopteris* extracts: Aqueous (SBA) and ethanolic (SBE)extract on the HepG2 cells. Cells were incubated with increasing concentrations(25-100 µg/ml) of SBA, SBE and Silymarin in culture medium for 24 hours. Results were expressed as the mean % of absorbance (ratio of absorbance in plant extracts treated and control wells) ± SEM of three independent experiments, each in triplicate and analyzed by one-way ANOVA. *****P < 0.05 compared to the control group.

Protective effect of Selaginella bryopteris (SB) against CCl_4- induced cellular damage: Fig. 3 (a) demonstrates the effect of SBA and SBE on CCl_4-induced *in vitro* cell damage. Results revealed that cell viability declined up to 18% in CCl_4 treated cell lines when compared with the normal control group. Conjoined treatment of SBE and SBA with CCl_4significantly increased the cell viability in dose-dependent manner when compared with CCl_4- treated cell line group. The cell viability was found to be 71% in SBE and 63% in SBA at 100 µg/mL when compared with the positive control silymarin.



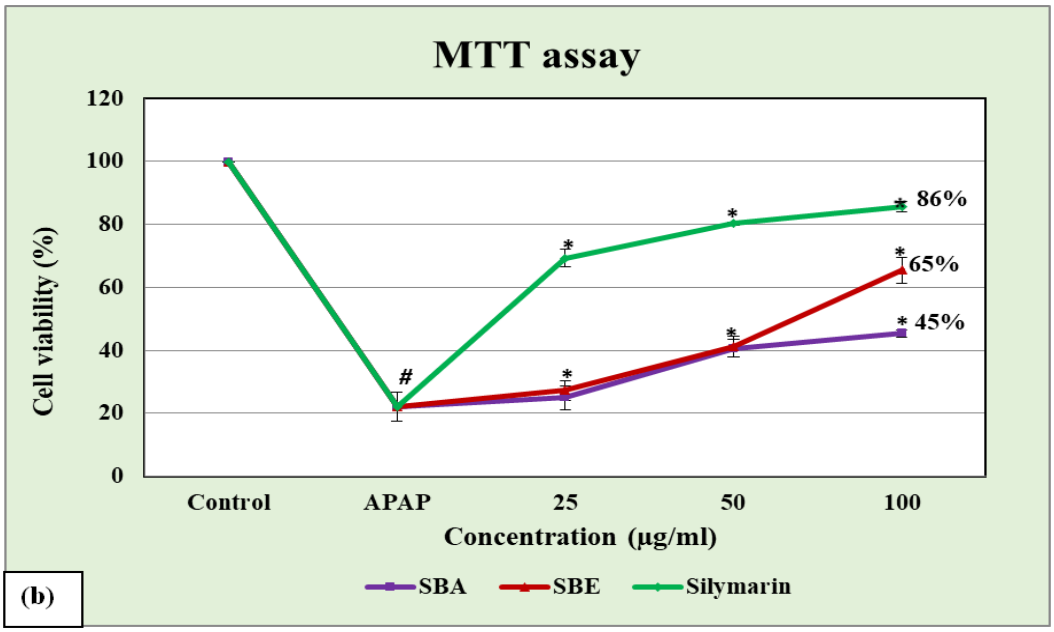


Figure 3: Effect of SBA and SBE on cell viability in CCl_4 and APAP treated HepG2 cell lines.

(a) Protective effect of *Selaginella bryopteris* extracts (SBA and SBE) against carbon tetrachloride (CCl_4) induced toxicity on HepG2 cell line. (b)Protective effect of *Selaginella bryopteris* extracts (SBA and SBE) against acetaminophen(APAP) induced toxicity on *HepG2* cell lines.

The results were expressed as the mean % of absorbance ± SEM of three independent experiments, each in triplicate. Values are mean ± standard error and analyzed by one-way ANOVA. *****P < 0.05 *vs* toxicant (APAP and CCl_4). **#** P<0.05 *vs* control.

***Protective effect of Selaginella bryopteris (SB) against APAP-induced cellular damage*:** Fig. 3 (b) depicts the cytoprotective effect of *Selaginella bryopteris* extract against APAP treated HepG2 cell line. APAP exposure in the HepG2 cell lines decreased (22%) cell viability rate which was improved by the administration of SB extract at three doses. The Maximum cell viability was found to be 65% at 100 µg/mL of SBE followed by 45% of the SBA against APAP- induced cell damage. The results were compared with the positive control silymarin which showed 86% cell viability at 100 µg/mL.

Discussion:

In the present study, the phytochemical constituents, total phenolic content and antioxidant activity were evaluated in the *Selaginella bryopteris* aqueous and ethanolic extracts. The hepatoprotective activity of SB extract was assessed in CCl_4 and APAP treated HepG2 cell line culture by MTT assay. The HepG2 cell culture technique has become one of the widely used cell lines used for screening of medicinal plant's properties including hepatoprotective activity (Hirsch C and Schildknecht S 2019). CCl_4 and APAP are common hepatotoxicants, which cause hepatic damage by generating free radicals through the liver's CYP450 enzyme system. Hence, they are widely used for screening of hepatoprotective plants (Ginting et. al., 2021). By cytochrome P-450 enzymes, CCl_4 is converted to trichloromethyl radical ($CCl3^{\cdot}$), a reactive metabolite, which reacts with the cell membrane components causing lipid peroxidation and finally leads to oxidative stress (González et al. 2017; Daoudi et al. 2021). The liver metabolizes acetaminophen via the cytochrome P450 pathway phase I (CYP2E1) into N-acetyl-p-benzo quinamine (NAPQI), which is then detoxified via the conjugation/sulfation reaction during phase II reaction. Overdose of APAP produces NAPQI at a higher level which binds to cellular components and further leads to

depletion of GSH level and simultaneously increases the level of lipid peroxidation and its consequences causes oxidative stress induced hepatic cell damage. (Yan et. al., 2018).

The magnitude of hepatic damage and protection of SB extract is determined in HepG2 cell lines. The MTT assay [3- (4,5 dimethyl thiazol-2yl)-2,5 diphenyl tetrazolium bromide] was used to determine the hepatoprotective effect of aqueous and ethanolic extracts of SB plant. It is based on the conversion of a yellow-coloured tetrazolium salt into blue-purple formazan by the mitochondrial succinate dehydrogenase enzyme (Berridge et al. 1996; Krithika et. al. 2013). MTT assay showed that the rate of cell viability was reduced (51%) in CCl_4 and APAP treated groups which were improved in SB extract treated HepG2 cell line group at three concentrations (25µg/mL, 50 µg/mL, 100 µg/mL). The enhancement of cell viability rate by the treatment of SB extract is due to its free radical scavenging activity. The cell viability rate was higher at 100 µg/mL of SB ethanolic extract followed by aqueous extract when compared with CCl_4 and APAP-treated HepG2 cell culture medium. The positive control, silymarin also showed similar protection when compared with the SB extract. The protection of hepatic cell lines against cellular damage may be attributed to the presence of phytochemical constituents which play an important role in the scavenging of free radicals produced by APAP and CCl_4. The SB treated alone HepG2 cells at all doses (25µg/mL, 50 µg/mL, 100 µg/mL) showed 99.9% cell viability indicating no adverse effect of SB extract when compared with control cells. The *in vitro* hepatoprotective effect of *Rauwolfiaserpentina* (Gnanasekaran et al. 2022) was found similar findings when compared with our *in vitro* results.

The ethanol extract of SB at 100µg/mL was found to have significant hepatoprotection followed by the aqueous extract of SB against CCl_4 and APAP-induced hepatic cell toxicity. SB aqueous and ethanol extract showed dose-dependent inhibition (17-89%) in DPPH, ABTS, and H_2O_2assays, underscoring its antioxidant activity which might be reduced the cellular damage caused by CCl_4 and APAP exposure (Ennaceria et al. 2019). In addition, the phytochemical analysis of SB aqueous and ethanolic extracts has shown the presence of flavonoids and phenolic compounds which have been known for their antioxidant and hepatoprotective activity (Vennila et al. 2021). Rupa and

Bhavani 2014; Chandrakant et. al. 2015; Singh et. al. 2016 also reported that *Selaginella bryopteris* has free radical scavenging activity and total phenolic content which were also found similar scavenging activities in our findings. The SB extract was found to have phenolic contents which may play an effective role in scavenging free radicals i.e., DPPH, ABTS and H_2O_2.

The results of the present study suggest that the ethanolic extract of *Selaginella Bryopteris* (SBE) have significant antioxidant and *in vitro* hepatoprotective activities followed by its aqueous extract against $CCl_4$and APAP induced toxicity in HepG2 cell lines. This protective effect of SB extract is due to the presence of free radical scavenging phenolic and flavonoid compounds. Thus, SB can serve as an alternative hepatoprotective agent. However, it is necessary to validate its hepatoprotective activities in *in vivo* model for further experimental study.

Conflicts of interest:

The authors declare no conflict of interest associated with this research work.

Acknowledgement:

The authors would like to thank School of Sciences, IGNOU and the School of Studies in Zoology, Jiwaji University, Gwalior (Madhya Pradesh) India for providing the facilities for this research work. The author Deena Prakash (Research scholar) is grateful to the Indira Gandhi National Open University, New Delhi (India) for providing Savitri Bai Phule Women Fellowship under the IGNOU-Research Fellowship scheme.

References:

Al-Manhel, A.J. and Niamah, A.K., (2015). Effect of Aqueous and Alcoholic Plant Extracts on Inhibition of Some Types of Microbes and Causing Spoilage of Food. Journal of Nutrition & Food Sciences. S5.

Antony, R. and Thomas, R., (2011). A mini review on medicinal properties of the resurrecting plant *Selaginella bryopteris* (Sanjeevani). International Journal of Pharmacy and Life sciences. 2(7), 933-939.

Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R., (1996). The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. Roche Applied Sciences. 14–19.

Chandrakant, J., Mathad, P., Mety, S., Ahmed, L., Sanaullah, (2015). Phytochemical and antidepressant activities of *Selaginella bryopteris* (l.) Baker on albino mice. International journal of applied biology and pharmaceutical technology. 6(4), 14-19.

Daoudi, Bnouham et al. (2021). Evaluation of toxicity, nephroprotective and hepatoprotective activities of Argan oil on CCl_4-induced nephrotoxicity and hepatotoxicity in Wistar rats. Arabian Journal of Medicinal and Aromatic Plants. 7(3), 438-464.

Ennacerie, F-Z et al. (2019). Polyphenols, antioxidant activity and mode of action of antimicrobial compounds of Dittrichia viscosa extracts. Arabian Journal of Medicinal and Aromatic Plants. 5(3), 90-106.

Ganeshaiah, K.N., Vasudeva, R., Shaanker, R,U., (2009). In search of Sanjeevani. Current Science. 97(4), 25.

Ginting, C, N., Lister, I, N, E., Girsang, E., Widowati, W., Yusepany, D, T., Azizah, A, M., Kusuma, H, S, W., (2021). Hepatotoxicity prevention in Acetaminophen-induced HepG2 cells by red betel (Piper crocatum Ruiz and Pav) extract from Indonesia via antioxidant, anti-inflammatory, and anti-necrotic. Heliyon. 7(1), e05620.

Gnanasekaran, A., Yukshna, A/P, R., Fouad, saleih, R.AL.,Murugesan K. et al. (2022). Evaluation on Antibacterial activity and hepatoprotectivity of Rauwolfia serpentina, Journal of Angiotherapy, 6(2), 620–631.

González, R.B., Minsky, N.W., Espinosa, L.E.M., Aranda, R.S., Mesequer, J.P., Perez, P.C., (2017). *In vitro* assessment of hepatoprotective agents against damage induced by acetaminophen and CCl4. BMC complementary and alternative medicine. 17, 39.

Harborne, J.B., (1998). Phytochemicalmethods: A guide to modern technique of plant analysis. London: Champman and Hall.

Hirsch, C., Schildknecht, S., (2019). *In vitro* Research Reproducibility: Keeping Up High Standards. Frontiers in pharmacology. 10,1484. doi: 10.3389/fphar.2019.01484

Imrana, Mohd., ASIF, Mohammad. (2020). Morphological, ethnobotanical, Pharmacognostical and pharmacological studies on the medicinal plant Plumeria alba linn. (apocynaceae). Arabian Journal of Medicinal and Aromatic Plants. 6(1), 54-84.

Jain, S., Mohan, R., Rai, R., (2015). Ocimum sanctum as an Herbal Medicine: A Review. International Journal of Oral and Maxillofacial Surgery. 1(1), 1-12.

Jannu, V., Baddam, P.G., Boorgula, A.K., Jambula, S.R., (2012). A Review on Hepatoprotective Plants. International Journal of Drug Development and Research. 3, 1- 8.

Jannuzzi, A.T., Kara, M., Alpertunga, B., (2018). Celastrol ameliorates acetaminophen-induced oxidative stress and cytotoxicity in Hep G2 cells. Human and Experimental Toxicology. 37(7), 742-751.

Jena, S., Ray, A., Rath, D., Sahoo, A., Singh, S., Nasim, N., et al. (2019). Curcuma angustifolia ameliorates carbon tetrachloride-induced hepatotoxicity in Hep G2 cells and Swiss albino rats. Asian Pacific Journal of Tropical Medicine. 12(9), 416-424.

Krithika, R., Verma, R.J., Shrivastav, P.S., (2013). Antioxidative and cytoprotective effects of andrographolide against CCl4-induced hepatotoxicity in Hep G2 cells. Human and Experimental Toxicology. 32(5), 530-543.

Kunert, O., Swamy, R.C., Kaiser, M., Presser, A., Buzzi, S., Appa-Rao, A.V.N., Schühly, W., (2008). Antiplasmodial and leishmanicidal activity of biflavonoids from Indian Selaginella bryopteris. Phytochemistry Letters. 1(4), 171-174.

Lowry, O.H., Rosebrough, N.J., Farr, A.I., Randall, R.J., (1951). Protein determination with the folin's phenol reagent. Journal of Biological Chemistry. 193, 265-275.

Mamri, Samira et al. (2022). Antioxidant properties and hepatoprotective effect of Croccus sativus stamens against carbon tetrachloride-induced liver injury in rats. Arabian Journal of Medicinal and Aromatic Plants. 8(3), 170-187.

Mishra, P.K., Raghuram, G.V., Bhargava, A., Ahirwar, A., Samarth, R., et al. (2011). *In vitro* and *in vivo* evaluation of the anticarcinogenic and cancer chemopreventive potential of a flavonoid-rich fraction from a traditional Indian herb Selaginella bryopteris. British Journal of Nutrition. 106, 1154–1168.

Mossmann, T., (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. Journal of Immunological Methods. 65, 55–63.

Owoyele, B.V., Adebukola, O.M., Funmilayo, A.A., Soladoy, A.O., (2008). Anti-inflammatory activities of ethanolic extract of Carica papaya leaves. Inflammo pharmacology. 16, 168–173.

Panda, V,S., Kharat, P.S,, Sudhamani, S., (2015). Antioxidant and hepatoprotective effect of Macrotylomauniflorum seed in antitubercular drug induced liver injury in rats. International journal of phytopharmacy. 4, 22-29.

Pandey, S., Khan, A.A., Shankar, K., Singh, N., (1999). An experimental study on the anti-stress and anti-oxidant of Selaginella bryopteris. Journal of Biological Chemistry. 12, 128–129.

Pareek, A., Godavarthi, A., Issarani, R., Nagori, B.P., (2013). Antioxidant and hepatoprotective activity of Fagoniaschweinfurthii (Hadidi) Hadidi extract in carbon tetrachloride induced hepatotoxicity in Hep G2 cell line and rats. Journal of ethnopharmacology. 150(3), 973-981.

Paswan, S.K., Gautam, A., Verma, P., Rao, V.C., Sidhu, O.P., Singh, A.P., Srivastava, S., (2017). The Indian Magical Herb 'Sanjeevni' (Selaginellabryopteris L.) -A Promising Anti-inflammatory Phytomedicine for the Treatment of Patients with Inflammatory Skin Diseases. Journal of Pharmacopuncture. 20(2), 093-109.

Re, R., Proteggente, P.N., Pannala, A., Ananth, R.E., Catherine, (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 26, 1231–1237.

Ruch, R., Cheng, S.J., Klaunig, J.E., (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis.10, 1003-1008.

Rupa, P. and Bhavani, N.L., (2014). Preliminary phytochemical screening of desiccated fronds of *Selaginella bryopteris* (L.) baker (pittakalu). World Journal of Pharmaceutical Research. 3(9), 1370-1378.

Sah, N. K., Singh, S. N. P., Sahdev, S., Banerji, S., Jha, V., Khan, Z., Hasnain, S. E., (2005). Indian herb Sanjeevani (Selaginellabryopteris) can promote growth and protect against heat shock and apoptotic activities of ultra violet and oxidative stress. Journal of Biosciences. 30(4), 499–505.

Sah, P., (2008). Does the Magical Himalayan Herb Sanjeevani Booti really exist in Nature? The Journal of Applied Sciences. 4(3), 65-67.

Setyawan, A.D. and Darusman, L.K., (2008). Review: Biflavonoid compounds of Selaginella P. Beauv. and its benefit. Biodiversitas. 9(1), 64-81.

Shakya, A.K., (2016). Medicinal plants: Future source of new drugs. International journal of herbal medicine. 4(4), 59-64.

Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., (1992). Antioxidative properties of Xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 40, 945–948.

Singh, R. S., Thakur, D., Jha, V. K., Kumar, U., Pal, A. K. et al. (2018). Antibacterial Activity of *Selaginella bryopteris* Fronds Extract on Bacteria Isolated from Mastititic Milk.

International Journal of Current Microbiology and Applied Science. 7, 4711-4715.

Singh, S. and Singh, R., (2015). A review on endemic Indian resurrecting herb *Selaginella bryopteris* (l.) Bak. Sanjeevani. International journal of pharmaceutical sciences. 6(1), 50-56.

Singh, S., Yogesh, J., Rawat, A.K.S., (2016). Evaluation of photochemical constituents of Cryptogamus of Almora District, Uttrakhand for their total phenolic content, total flavonoids and antioxidant potential. International Journal of Research in Pharmacy and Chemistry. 6(4), 833-842.

Soldatow, V. Y., LeCluyse, E. L., Griffith, L. G., Rusyn, I., (2013). *In vitro* models for liver toxicity testing. Toxicology Research. 2(1), 23–39.

Tripathi, K, D., (2013). Essentials of Medical Pharmacology. 7th ed. Delhi.

Vennila, K., Arunprasath, A (2021). Evaluation of 2,2-diphenyl-1-picrylhydrazyl, secondary metabolite contents and antimicrobial efficacy of Blastonia garcinii(Burm.f.) Cogn. Arabian Journal of Medicinal and Aromatic Plants. 7(2), 227-237.

Verma, M., Gangwar, M., Sahai, M., Singh, T. D., (2015). Antimicrobial Activity of Phytochemicals Isolated from Selaginella bryopteris. Chemistry of Natural Compounds. 51(2), 341-345.

Wang, X., Qinghua, W., Aimei, L., Arturo, A., José-Luis, R., (2017). Paracetamol: overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds in vivo and *In vitro*. Drug Metabolism Reviews. 49(4), 395-437.

World Health Organization report, (2019). A report.

Yan, M., Huo, Y., Yin, S., Hu, H. (2018). Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. Redox Biol. 17, 274-283. doi: 10.1016/j.redox.2018.04.019.

Arabian Journal of Medicinal and Aromatic Plants

www.ajmap.info **ISSN 2458-5920**