Research Article
ISSN: 2249-1236
CODEN: IJRRBI (USA)
Naveen Sharma *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci.,*(2018) 8(1) 069-082
International Journal of Research and Reviews in Pharmacy and Applied sciences

Radical scavenging potential and Hepatoprotective studies of ethanolic and aqueous extract of *Actiniopteris Dichotoma Bedd* against Paracetamol Diclofenac and CCl_4 induced hepatotoxicity

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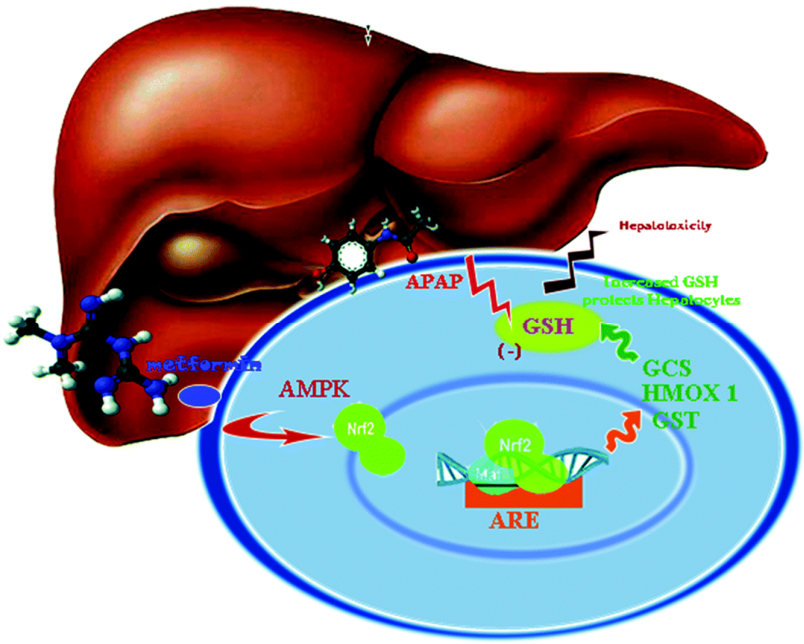


Abstract:

This study was carried out to investigate the free radical scavenging potential and hepatoprotective activity of the ethanolic and aqueous extract of the Actiniopteris dichotoma using paracetamol, Diclofenac and CCl_4 hepatotoxicants. Extracts were used for estimation of radical scavenging potential of the plant. Plant extracts at the dose of 250 and 500 mg/kg, p.o. and silymarin as a standard drug were administered in intoxicated rats and hepatoprotective potential assessed by different biochemical parameters like AST, ALT, ALP, total protein, bilirubin, and albumin content by UV kinetic assay. At the dose of 250mg/kg, p. o. offered significant ($P<0.05$) but dose 500mg/kg, p. o. offered significant ($P<0.01$) compared with that of standard silymarin ($P<0.001$). Ethanolic and aqueous extract of the Actiniopteris dichotoma bedd showed hepatoprotective effect against paracetamol, diclofenac and CCl4- induced hepatotoxicity, which was evidenced by the significant decrease in ALT, AST and ALP. On the light of the present study it is concluded that the ethanolic and aqueous extract of the selected plant could protect liver against oxidative damages and could be used as an effective protector against hepatotoxicants induced hepatic damages.

Keywords: Antioxidant, Hepatoprotective, Actiniopteris dichotoma bedd, Diclofenac, Paracetamol, CCl_4.

Introduction:

The liver is the largest gland of the body which perform more than 500 vital metabolic and biochemical functions.[1] It is involved in the synthesis of products like glucose derived from glycogenesis, plasma proteins, clotting factors and urea that are released into the bloodstream. It regulates blood levels of amino acids. Liver cell serves as a storage organ for several products like glycogen, fat and fat soluble vitamins. Hepatocytes cells are also involved in the production of a substance called bile that is excreted to the intestinal tract. Bile helps in the removal of toxic substances and serves as a filter that separates out harmful substances from the bloodstream and excretes them[2]. It plays an important role in drug elimination and detoxification and liver damage may be caused by xenobiotics, malnutrition, alcohol consumption, medications, anaemia infection and medications.[3] Hepatotoxicity is a injury of the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent.[4] Liver plays a important role in regulating various physiological processes in the body that is why it is the most important organ of the body, which involved in several vital functions such as metabolism, secretion and storage. The world health organization (WHO) has recently defined traditional and herbal medicine as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The utilisation of plants in the treatment of many human diseases is evidence of man's ingenuity. The contribution of plants to the therapeutic armaments in the fight against disease dates back several centuries, and has, to a certain extent, been documented by the ancient Chinese, Indian, Japan and North African civilisations. Currently, traditional medicine is widely practiced, especially in developing countries. Traditional herbal medicines are naturally occurring; plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. Herbal medicines are playing an important role in health improvement program in the world, and there is resurgence of interest in herbal drugs for treatment of various ailments, India, the abode of Ayurvedic system of medicine, assigns much importance to the pharmacological aspect of many plants. Traditional herbal medicines are getting significant attention in global health debates.[5] The title plant *Actiniopteris dichotoma bedd* is used in folk medicine for treatment of various types of diseases. The tribals are familiar about the medicinal uses of *Actiniopteris dichotoma bedd* plant found in their village surroundings and forest areas. This plant has been used as folk and traditional medicine in the treatment of various hazardous diseases. [6]

Materials and Methods:

1. Collection and Authentication:

The plant was collected from Chitrakoot, Satana, Madhya Pradesh in the rainy season of July and August. The plant was authenticated by NISCAIR near Pusa gate, New Delhi, India. Reference No. NISCAIR/RHMD/Consult/2010-11/1408/06.

2. Extraction of Plant:

The plant was dried and powdered. A fine coarse powder was obtained which was sieved through #40 to obtain uniformity. The powered drug were extracted with distil water and ethanol solvent. Continues soxhlet extraction method was used, the powder of crude drug was packed in a thimble made whatman filter paper and inserted in to the extractor. Each batch extracted for about 35 cycles. The extracts were then made to powder by using rotary evaporator under reduced pressure. When the extraction was completed, the extractant concentrated under vacuum, for large volumes and by heating at low temperature. Aqueous extracts were generally freeze-dried and stored at 20^{o}C as this low temperature reduces the degradation of the bioactive natural product.

3. In-vitro radical scavenging potential:

3.1-DPPH radical scavenging activity:

Solution of DPPH (400 μM) in 100% ethanol was prepared and 1 ml of this solution added with 3 ml of the extract dissolved at different concentration (10, 20, 40, 60, 80 and 100 μg/ml).The mixture was shaken and allowed to stand at room temperature for 30 minutes. After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured by reading the absorbance at 517nm using UV-Visible Spectrophotometer. Initially,

absorption of blank sample containing the same amount of ethanol and DPPH solution was prepared and measured as control. Ascorbic acid was used as standard. The IC_{50} values of the crude extract and standard ascorbic acid were calculated.

3.2-Nitric oxide scavenging activity:[7]

2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations prepared in ethanol and the mixture incubated at 25°C for 30 min. Thereafter, 1.5ml of Griess reagent (Griess reagent contains 0.2% naphthyl ethylene diamine dihydrochloride, and 2% sulphanilamide in 5% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard.

3.3-Determination of reducing power: [8]

Various concentrations of the extracts (2g/ mL) in 1.0 mL of deionized water were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 500°C for 20 minutes; aliquots of trichloroacetic acid (2.5 mL, 10%) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Reducing power is given in ascorbic acid equivalent (ASE mL^{-1}) that shows the amount of ascorbic acid expressed in mM.

4. Evaluation of Hepatoprotective Activity:

4.1-Doses and Treatments:

Silymarin 100 mg/kg .p.o was used as standard drugs. Plant extract ethanolic at two different doses (250, 500 mg/kg) were administered. The control group received 0.9 % saline or Sterile distilled water, 5 ml/kg, p o as vehicle.

4.2-Paracetamol induced hepatotoxicity:[9]

The mice were divided into five groups with six mice in each. Group I (Negative Control) served as normal and received Sterile distilled water, 5 ml/kg, p.o. Paracetamol was administered as a single dose of 250 mg/kg to the all Group. After 48 h, they were treated with the test drug for 7 days. Group II served as positive control received normal saline 5 ml/kg and group III served as standard, treated with silymarin (100 mg/kg p.o.), group IV,V were treated with aqueous and ethanolic extract of *Actiniopteris dichotoma bedd* at a dose level of 250 mg/kg and 500 mg/kg body weight p.o. per day respectively for 7 days.

4.3-Diclofenac induced hepatotoxicity:

The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 5 ml/kg, p.o.) for 7 days. Group II served as positive control received normal saline 5 ml/kg and group III served as standard animals received silymarin (100 mg/kg p.o.) for 7 days. Group IV and V were treated with aqueous and ethanolic extract of Actiniopteris dichotoma at a dose level of 250 mg/kg and 500 mg/kg body weight p.o. per day respectively for 7 days and on the 3rd and 4th day diclofenac (50 mg/kg i.p.) was given 1 h after the treatment of the extract.

4.4-Carbontetrachloride-induced hepatotoxicity:[10]

The animals (Rats) were divided into five groups of five animals each. Group-I served as negative control received sterile distilled water, 5 ml/kg, p.o.) daily once for 7 days. Group-II served as positive control and received CCl_4 (1 ml/kg i.p) daily once for 7 days. Group-III was treated with the standard drug Silymarin (100 mg/kg .p.o) and followed by CCl_4 (1 ml/kg i.p) daily once for 7 days. Groups IV-V were treated aqueous and ethanolic extract of *Actiniopteris dichotoma* at different doses (250 mg/kg.b.wt and 500 mg/kg.b.wt.) respectively followed by CCl_4 (1 ml/kg i.p) daily once for 7 days.

4.5-Collection of Blood Samples:
All the animals were sacrificed on 7th day under light ether anaesthesia. The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at 37°C .The clear serum was separated at 2500rpm (Microcentrifuge) for 10min and subjected to biochemical investigation.

4.6-Assessment of Liver Function:
The serum aspartate aminotransaminase (AST)[11] or Serum glutamic oxaloacetic transaminase (SGOT) and alanine aminotransferase **(**ALT)[12] or serum glutamic pyruvic transaminase (SGPT), ALP[13,14] Total Protein Assay[12], Bilirubin Assay[15,16], Assay of albumin content[17], assay of triglyceride, assay of Cholesterol were estimated by UV kinetic assay with the help of test kit (Liquid Gold and Autospan).

5-Statistical Analysis:
Results of biochemical estimation were reported as mean ±SEM for determination of significant inter group difference was analyzed separately and one-way analysis of variance (ANOVA) was carried out[18]. Dunnet's test was used for individual comparisons.[19]

6. Results and Discussion:
6.1-Evaluation of In-vitro radical scavenging potential:
6.1.1-DPPH free radical scavenging activity:
In DPPH method, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The results of absorbance and % inhibition showed decrease in concentration of DPPH radical due to scavenging ability of extract and standard ascorbic acid, as a reference compound. The ethanolic extract presented the better activity at all concentrations when compared to other extract. A 100 µg/mL of ethanol extract, aqueous extract, acetone extract and ascorbic acid exhibits 85.24%, 74.33%, 51.25% and 94.4% inhibition respectively and the IC_{50} values were found to be 36.81 µg/mL, 88.50 µg/mL, 128.40 µg/mL and 8.57 µg/mL for aqueous, ethanolic extract and ascorbic acid, respectively.

6.1.2-Nitric oxide radical scavenging activity:
The nitric oxide generated from sodium nitroprusside and reacts with oxygen to form nitrite, which is inhibited by antioxidants. Sulfanilamide is quantitatively converted to a diazonium salt by reacting with nitrite in acidic conditions (5% phosphoric acid). This diazonium salt coupled with N- (1- naphthyl)-ethylenediamine (NED); forming an azo dye that can be measured quantitatively at 542 nm. The results showed that the ethanolic extract has higher % inhibition and lowest IC_{50} value as compared to other extract. The % inhibition and IC_{50} values of ethanolic extract, aqueous extract, acetone extract and ascorbic acid were 79.75 %, 62.97 %, 47.68 % and 95.27 % and 60.50 µg/mL, 87.02 µg/mL, 107.02 µg/mL and 10.82 µg/mL respectively.

6.1.3-Reducing power activity:
The reducing capability of a compound may serve as a significant indicator of its potential antioxidant. The reducing power of extracts increased with increasing concentration. The results showed that ethanolic extract has higher reducing power as compared to other extract. Ethanol and aqueous extract exhibited the higher reducing power as compare to the acetone extract. This result indicates that the extracts may consist of antioxidant compounds that usually show great reducing power. This has been justified by ethanol and aqueous extract being the most reducing agent.

Various concentrations ranging from 10-100 µg/ml of extracts were tested for their free radical scavenging activity it was observed that free radical scavenged by whole plant extracts is in dose dependent manner. Ethanol, aqueous and acetone extract of *Actinioperis dichotoma bedd* were detected and compared with Ascorbic acid. The IC_{50} values for DPPH assay of for ethanol extract was found maximum followed by aqueous extract and for acetone extract was

minimum. Though the extracts showed good DPPH scavenging activity but it was less effective than standard Ascorbic acid. The Nitric oxide scavenging activity of Ethanol, aqueous and acetone extract of *Actinioperis dichotoma bedd* were detected and compared with Ascorbic acid. The IC_{50} values for nitric oxide scavenging activity of for ethanol extract was found maximum followed by aqueous extract and for acetone extract was minimum. Though the extracts showed good nitric oxide activity but it was less effective than standard Ascorbic acid.

6.2-Evaluation of Hepatoprotective activity:

6.2.1-Paracetamol induced Hepatotoxicity:

Effect of Actiniopteris dichotoma on liver enzyme markers Biochemical analysis of liver enzyme markers signified that the use of paracetamol in high doses strikingly raised the serum levels of liver enzyme markers ALT, AST, ALP and also serum level as compared to the control group. Treatment with ethanolic and aqueous extract of Actiniopteris dichotoma showed hepatoprotective activity against paracetamol induced hepatotoxicity by maintaining the serum levels of ALT, AST, and ALP at significantly ($P < 0.05$) , ($P < 0.01$) reduced levels in a dose dependent manner. Treatment with plant extract decreased serum levels of bilirubin significantly when directly compared with paracetamol treated mice. Results showed in table no.1.The results depicted in table no.2 indicate that free fatty acids, cholesterol, Triglycerides and phospholipids in serum of the paracetamol treated groups were elevated. After the treatment with plant extract a decrease significantly ($P< 0.05$), ($P < 0.01$) in the serum cholesterol, triglycerides, phospholipids and free fatty acids was evident which was comparable with silymarin treated groups.

In the hepatocytes, AST and ALP were present in high concentration. These enzymes are released from the cells and their levels in the serum increases due to hepatocyte necrosis or aberrant membrane permeability. ALT is a sensitive indicator of the acute liver damage and elevation of this enzyme in non-hepatic disease is unusual. ALT is more selectively a liver parenchymal enzyme than AST indicating the extent of liver damage. In the present study the elevation of the enzymes in the serum may be due to the damage of membrane and the subsequent leakage of the enzymes in to serum. Treatment with the plant extracts were improved the membrane integrity, decreasing the enzymes levels in serum.

6.2.2-Diclofenac Sodium induced hepatotoxicity:

Serum biochemical study Serum activities of transaminases, AST, ALT, GGT and ALP were given in Table no. 3. Single dose of DIC elevated AST, ALT and GGT activities when compared to the normal animals. Treatment of ethanol extract of Actiniopteris dichotoma 1 h prior to DIC (50mg/kg) administration significantly protected the elevation of transaminases and ALP activities. The activities of AST, ALT and GGT in the ethanolic extract of Actiniopteris dichotoma (500 mg/kg) plus DIC treated group were significantly reduced respectively. But the aqueous extract of Actiniopteris dichotoma also less significantly reduced as compare to the standard drug.

The ALT, AST, ALP and GGT levels were significantly elevated when rats were administered with diclofenac indicating hepatocellular damage. The increased levels of these enzymes were significantly decreased by treatment with plant extract in dose dependent manner. This is the indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by diclofenac.

6.2.3-Carbon tetrachloride induced hepatotoxicity:

All the marker enzymes AST, ALT, ALP, GGT and total bilirubin were significantly increased($^a P < 0.001$) in CCl_4 treated rats as compare to control group but the total protein level significant decreased ($^a P < 0.001$) reflecting the liver injury caused by CCl_4 and also cholesterol level significant increase ($^a P<0.001$). Ethanolic and aqueous extract of *Actiniopteris dichotoma bedd* at the higher dose (500 mg/kg, p o) showed significant reduction ($P < 0.01$) in the level of serum markers and significant increase ($P < 0.01$) in total protein and albumin to the near normal which are comparable to the values registered in the standard drug treated group of animals, indicating the protection of liver cells. Extract of plant at lower dose (250mg/kg, po) showed significant reduction ($P < 0.05$) in the level of serum

markers and significant increase ($P < 0.05$) in total protein and albumin. The activity of the extracts is found to be dose dependant.

The extent of hepatic damage is assessed by the elevated level of biochemical parameters, which attributed to the generation of trichloromethyl free radical which in turn causes peroxidation of lipids in the cellular membrane. All the marker enzymes AST, ALT, ALP, GGT and total bilirubin were significantly increased in CCl_4 treated rats as compare to control group but the total protein level significant decreased reflecting the liver injury caused by CCl_4 and also cholesterol level significant increase. Whereas blood samples from the animals treated with ethanolic and aqueous extract of *Actiniopteris dichotoma bedd* at the higher dose (500mg/kg, po) showed significant reduction in the level of serum markers and significant increase in total protein and albumin to the near normal which are comparable to the values registered in the standard drug treated group of animals, indicating the protection of liver cells. But both extract of plant at lower dose showed significant reduction in the level of serum markers and significant increase in total protein and albumin.

7. Conclusion:

The present study depicts the efficacy of the plant extracts in activating the protective systems of the animal there by restoring the normal functioning of the liver. It is clear from the study that the plant possesses significant free radical scavenging and hepatoprotective potential. Paracetamol, Diclofenac and CCl4 induced hepatotoxicity models, the serum biochemical parameters and liver antioxidants were altered when animals were intoxicated with paracetamol, diclofenac and CCl4. The level of serum biochemical parameters were restored with the treatment of extracts of plant.

Table.1 DPPH radical scavenging activity of plant extract in ethanol, aqueous and acetone solvent system

Extract	Concentration (µg/ mL) and % inhibition						IC 50 (µg/mL)
	10	20	40	60	80	100	
Std. Ascorbic Acid	42.6 ± 1.68	53.6 ± 1.50	58.5 ± 1.22	66.1 ± 0.28	76.23 ± 1.20	91.4± 0.47	8.57 ± 0.24
Ethanolic extract	31.74 ± 2.39**	34.22 ± 2.57**	39.05 ± 1.18**	44.07± 1.28**	59.14± 1.36**	85.24± 1.56**	36.81 ± 2.28
Aqueous extract	30.14 ± 1.49	32.28 ± 1.17	35.03 ± 1.28*	41.12± 2.18*	52.14± 1.26*	74.33± 2.46**	88.50 ± 1.28
Acetone extract	25.14 ± 1.33	28.22 ± 2.97	31.03 ± 1.24*	37.12± 1.34	43.14± 2.16	51.25± 1.26	128.40 ± 1.28

Values are given as mean ± S. D. (n=3). ** Significant at $p < 0.01$, *Significant at $p < 0.05$ p-value was calculated by comparing with control by ANOVA followed by Dunnett's test, values are expressed as ± SEM.

Table.2 Nitric oxide radical scavenging activity of plant extract in ethanol, aqueous and acetone solvent system

Extract	Concentration (µg/ mL) and % inhibition						IC 50 (µg/mL)
	10	20	40	60	80	100	
Standard Ascorbic Acid	41.6 ± 0.28	52.5 ± 0.51	65.5 ± 0.32	75.2 ± 0.28	88.11 ± 1.22	95.27 ± 1.27	10.82 ± 1.21
Ethanolic extract	30.34 ± 2.39**	32.12 ± 2.17**	36.15 ± 1.28**	47.07± 0.18**	57.12± 1.26**	79.75 ± 1.16**	60.50 ± 1.18
Aqueous extract	28.12 ± 2.19	30.18 ± 1.02*	33.13 ± 0.12*	42.12± 2.18*	52.11± 1.16*	62.97 ± 1.42**	87.02 ± 1.12
Acetone extract	22.12 ± 1.29	26.12 ± 1.47	30.02 ± 1.14	34.15± 1.31	41.12± 1.12	47.68 ± 2.21	107.02 ± 2.28

Values are given as mean ± S. D. (n=3). ** Significant at $p < 0.01$, *Significant at $p < 0.05$ p-value was calculated by comparing with control by ANOVA followed by Dunnett's test, values are expressed as ± SEM.

Table.3 Reducing power activity of plant extract in ethanol, aqueous and acetone solvent system

Concentration (µg/mL)	Absorbance			
	Ascorbic acid	Ethanolic Extract	Aqueous Extract	Acetone Extract
100	0.62 ± 0.06	0.28 ± 0.04**	0.09 ± 0.02*	0.04 ± 0.01
200	0.92 ± 0.02	0.69 ± 0.05**	0.26 ± 0.04**	0.12 ± 0.04
400	1.74 ± 0.03	0.98 ± 0.01**	0.49 ± 0.05**	0.29 ± 0.07
600	1.99 ± 0.08	1.25 ± 0.06**	0.58 ± 0.08*	0.38 ± 0.05
800	3.24 ± 0.02	1.89 ± 0.04**	0.95 ± 0.07**	0.45 ± 0.06
1000	3.82 ± 0.03	2.01 ± 0.06**	1.11 ± 0.02*	0.91 ± 0.04

Values are given as mean ± S. D. (n=3). ** Significant at $p < 0.01$, *Significant at $p < 0.05$ p-value was calculated by comparing with control by ANOVA followed by Dunnett's test, values are expressed as ± SEM.

Table.4: Effect of ethanolic and aqueous extract of Actiniopteris dichotoma bedd on different liver biomarkers with paracetamol administration

Groups	Dose Mg/kg	AST (IUL^{-1})	ALT (IUL^{-1})	ALP (IUL^{-1})	T/P (gL^{-1})	ALB (gL^{-1})	Serum bilirubin (mg/dl)
Normal Control	5ml	78.23 ± 0.15	34.11 ± 3.11	132.18 ± 1.12	53.15 ± 2.11	30.42 ± 1.17	0.29±0.09
Paracetamol	500	138.34 ± 2.45[a]	118.34 ± 4.13[a]	162.26 ± 3.20[a]	98.15 ± 4.17[a]	71.54 ±0.22[a]	1.86±0.44[a]
Silymarin	100	81.13 ± 1.12[#]	36.12 ± 2.12[#]	133.14 ± 1.11[#]	54.12 ± 2.12[#]	31.22 ± 2.23[#]	0.26±0.08[#]
EEAD	250	87.31 ± 2.14[*]	41.16 ± 2.15[*]	144.16 ± 3.14[*]	59.13 ± 1.14[*]	38.15 ± 0.34[*]	0.98±0.02[*]
EEAD	500	81.01 ± 0.14[***]	36.14 ± 1.11[***]	135.12 ± 0.11[***]	56.14 ± 1.14[**]	33.02 ± 0.11[**]	0.46±0.01[***]
AEAD	250	96.04 ± 0.12[*]	46.26 ± 2.21[*]	150.14 ± 2.11[*]	65.11 ± 1.01[*]	41.26 ± 2.01[*]	0.99±0.06[*]
AEAD	500	83.11 ± 2.11[**]	38.14 ± 2.10[**]	139.11 ± 1.21[**]	61.11 ± 1.14[*]	39.12 ± 4.21[*]	0.61±0.05[**]

All values are expressed as mean ± SEM (n=5), One-way ANOVA followed by Dunnett's test. [a](P < 0.01) compared with normal control, [*](P < 0.05) significant compared with treated control, [**](P < 0.01) and [***, #] (P < 0.001) significant compared with paracetamol treated control.

Table.5: Effect of ethanolic and aqueous extract of Actiniopteris dichotoma bedd on free fatty acid, Cholesterol, Triglycerides and Phospholipids with paracetamol administration

Groups	Dose (mg/kg)	Free Fatty Acid (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipid (mg/dl)
Normal Control	5ml	8.57±0.15	143.32±1.02	151.4±2.43	94.01±0.14
Paracetamol Treated	500	17.82± 0.81[a]	259.18±0.16 [a]	193.33±1.19 [a]	142.91±0.46 [a]
Silymarin	100	8.65± 1.1[#]	144.49± 0.05[#]	141.16± 0.46[#]	101.21± 0.51[#]
EEAD	250	12.10± 2.03*	164.17±1.05*	156.67±0.26*	124.61± 1.22
EEAD	500	8.53± 0.42**	141.67±1.16**	138.33± 1.53**	99.03± 0.26**
AEAD	250	12.35± 1.03*	169.29± 1.4*	158.93± 2.06*	131.61± 1.21
AEAD	500	9.10± 1.01**	146.41± 0.05**	146.16± 0.72*	112.8± 0.21*

All values are expressed as mean ± SEM (n=5), One-way ANOVA followed by Dunnett's test. [a](P < 0.01) compared with normal control, [*](P < 0.05) significant compared with treated control, [**](P < 0.01) and [#] (P < 0.001) significant compared with treated control.

Table.6: Effect of ethanolic and aqueous extract of Actiniopteris dichotoma on biomarkers with Diclofenac (DIC) administration.

Groups	Dose Mg/kg	AST (1 U/L Serum)	ALT (1 U/L Serum)	ALP (1 U/L Serum)	GGT (1 U/L Serum)
Control	5ml	71.86 ± 8.35	35.97 ± 2.83	118.23 ± 7.29	2.34±0.92 1
Diclofenac	50	125.96 ± 10.93	112.12 ± 6.52	246.85 ± 11.81	7.08±0.41
Silymarin	100	75.86 ± 8.35#	37.97 ± 2.83#	122.23 ± 7.29#	2.76±0.92#
EEAD	250	87.11 ± 5.40*	65.70 ± 4.18*	153.91 ± 5.18*	2.87±0.81*
EEAD	500	83.11 ± 9.40**	59.70 ± 5.48**	143.91 ± 5.18**	2.87±0.81**
AEAD	250	98.01 ± 5.14*	91.32 ± 2.24*	195.26 ± 4.01*	4.02±0.36*
AEAD	500	94.61 ± 6.28*	81.64 ± 3.57*	185.28 ± 7.02*	3.05±0.38*

All values are expressed as mean ± SEM (n=5), One-way ANOVA followed by Dunnett's test. *(P < 0.05), **(P < 0.01) #(P < 0.001) significant decreased compared with normal control.

Table.7: Effect of ethanolic and aqueous extract of Actiniopteris dichotoma on biomarkers in rats with CCl_4 administration

Groups	Dose Mg/kg	AST (1 U/L Serum)	ALT (1 U/L Serum)	ALP (1 U/L Serum)	GGT (1 U/L Serum)
Control	5ml	20.25±2.8	25.52±2.34	76.02±4.52	3.2±0.51
CCl_4	800	42.51±1.34[a]	65.82±2.68 [a]	138.14±6.2 5 [a]	28.25±0.18 [a]
Silymarin	100	22.24±3.8#	26.42±1.25#	79.14±2.6#	5.55±0.22#
EEAD	250	29.42±0.56*	34.43±1.5*	96.15±3.8*	10.55±7.9*
EEAD	500	24.82±0.56**	28.63±2.4**	84.16±3.8**	8.05±8.9**
AEAD	250	29.92±4.56*	35.83±0.4*	99.14±3.8*	12.55±6.4*
AEAD	500	25.02±2.66**	29.11±5.4**	87.14±2.8**	9.55±1.8**

Values were expressed as mean ±SEM (n = 5), [a] P < 0.001 significant difference from normal control, P < 0.05*, P < 0.01** P < 0.001# significant difference from CCl_4 treated control.

Table.8: Effect of ethanolic and aqueous extracts of Actiniopteris dichotoma bedd on biomarkers with CCl_4 administration

Groups	Dose Mg/kg	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)
Control	5ml	6.82±0.41	3.98±0.16	0.81 ± 0.06
CCl_4	800	3.02±0.12 [a]	1.06±0.24 [a]	2.14 ± 0.03 [a]
Silymarin	100	6.01±0.4**	3.88±0.12***	0.86 ±0.04***
EEAD	250	4.42±0.36*	4.35±0.24	1.54 ± 2.42*
EEAD	500	5.98±0.33***	3.34±0.32**	1.00 ±1.43**
AEAD	250	4.25±0.32*	4.12±0.25*	1.78 ± 3.26*
AEAD	500	5.68±0.34***	3.12±0.32**	1.06 ±1.07**

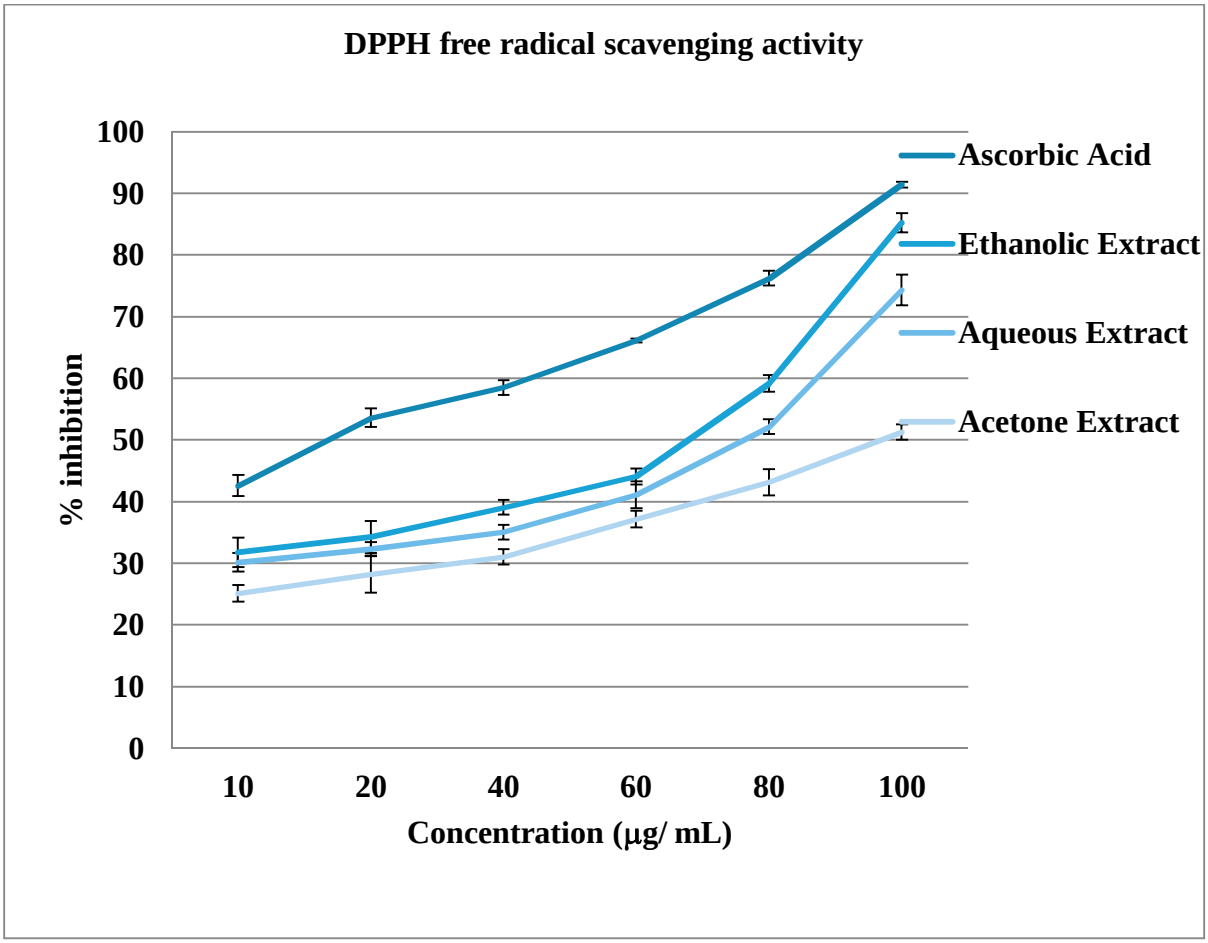
Values were expressed as mean ±SEM (n = 5), [a] P < 0.001 significant difference from normal control, P < 0.05*, P < 0.01** P < 0.001*** significant difference from CCl_4 treated control.

Table.9: Effect of ethanolic and aqueous extract of Actiniopteris dichotoma bedd on biomarkers in rats with CCl_4 administration

Groups	Dose (mg/kg)	Total Lipids (mg/100ml)	Triglycerides (mg/100ml)	Cholesterol (mg/100ml)	Phospholipid (mg/100ml)
Normal Control	5ml	133.45±5.15	9.2±0.82	68.94±2.61	122.01±5.92
CCl_4-Treated	800	270.82±9.2 [a]	15.14±2.14 [a]	100.02±4.2 [a]	280.82±15.22 [a]
Sylimarine	100	140.33±6.2***	9.3±0.90***	69.02±2.10***	125.15±6.98***
EEAD	250	151.24±8.41*	11.2±0.25***	78.12±4.01*	139.52±8.13*
EEAD	500	142.23±5.21**	10.2±0.51**	70.82±2.01**	130.52±9.33**
AEAD	250	159.24±7.11*	11.6±0.44*	79.82±4.02*	145.12±5.31*
AEAD	500	146.14±2.42**	10.6±0.55**	71.62±1.04**	133.22±4.12**

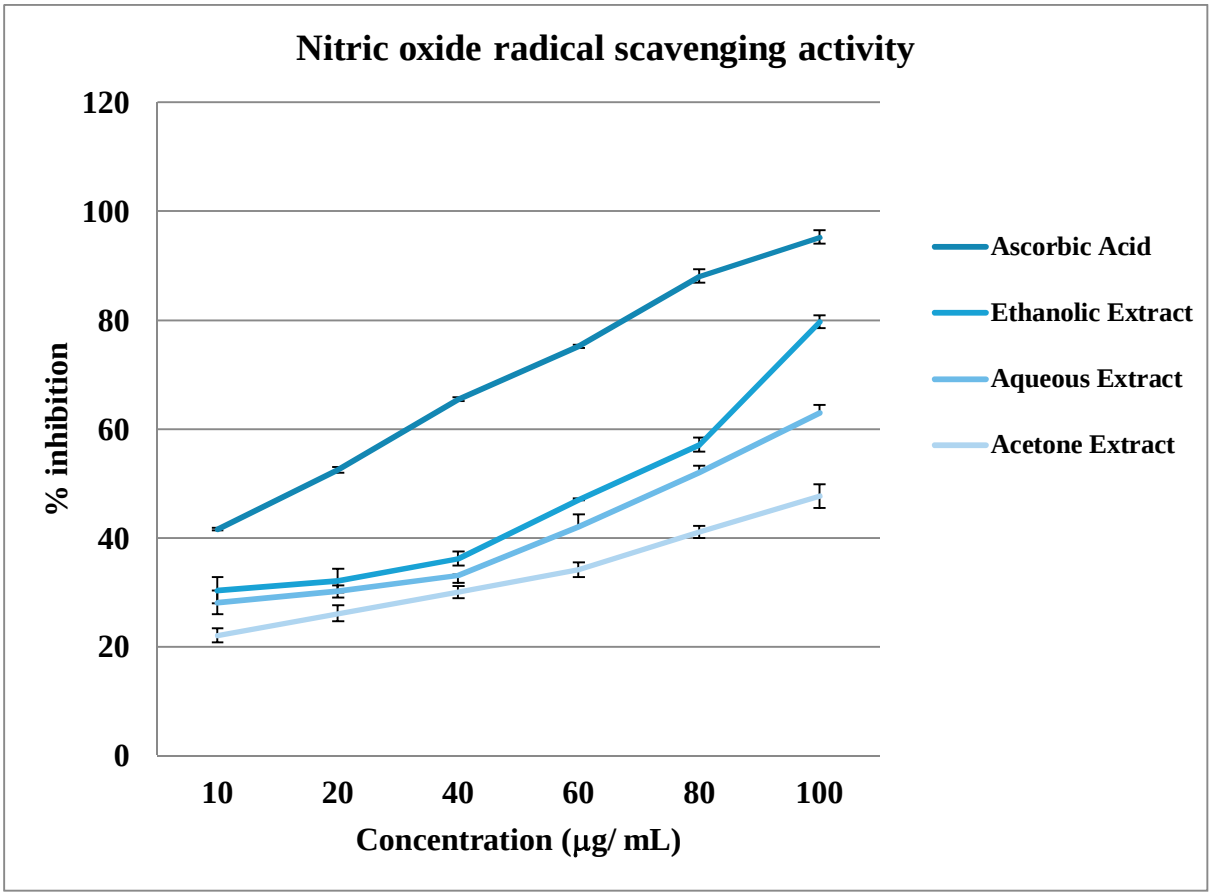
Values were expressed as mean ±SEM (n = 5), [a] P < 0.001 significant difference from normal control, P < 0.05*, P < 0.01** P < 0.001*** significant difference from CCl_4 treated control.

Graph.1: DPPH radical scavenging activity of plant extract in ethanol, aqueous and acetone solvent system



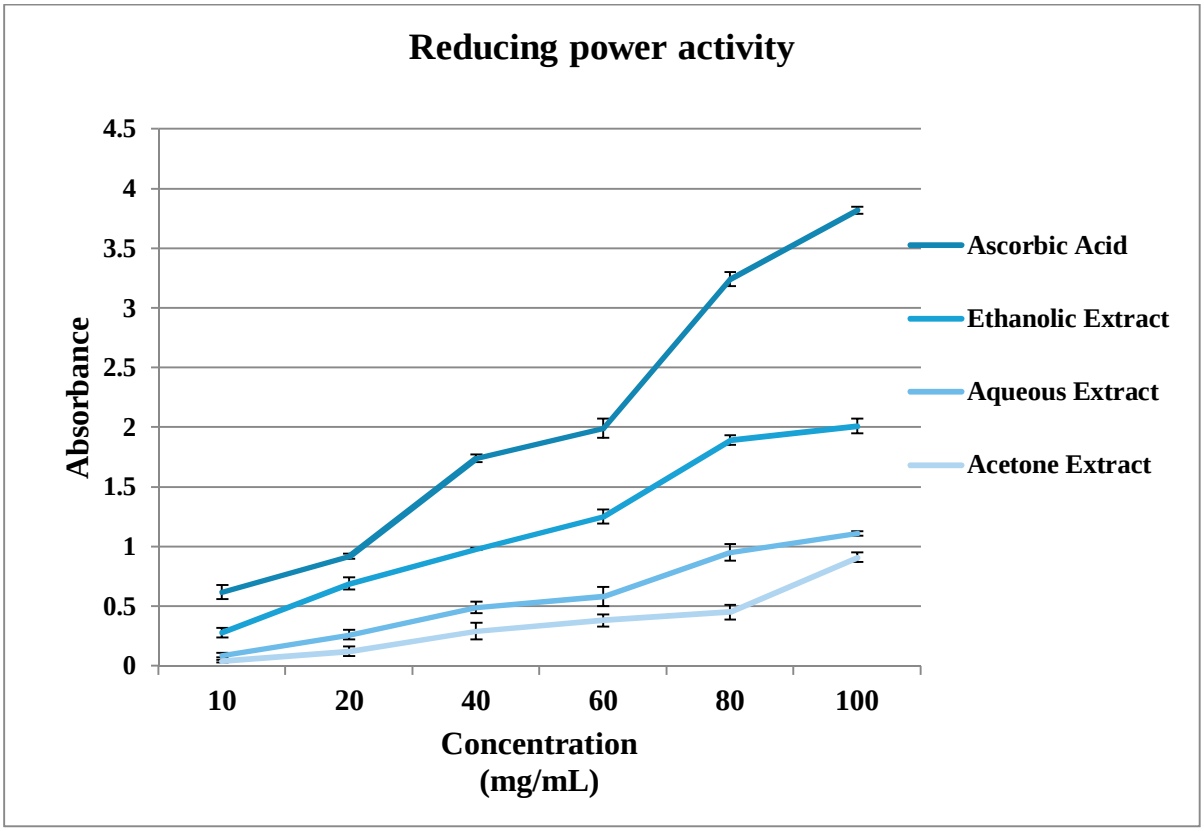
The graph shows *In-vitro* concentration dependent percentage inhibition of DPPH radical by ethanolic, aqueous and acetone extract of *Actiniopteris dichotoma Bedd.* In this graph plant extract shows percentage inhibition in all three solvent but ethanolic extract present better activity at all concentration.

Graph.2: Nitric oxide radical scavenging activity of plant extract in ethanol, aqueous and acetone solvent system



The graph shows *In-vitro* concentration dependent percentage inhibition of nitric oxide radical by ethanolic, aqueous and acetone extract of *Actiniopteris dichotoma Bedd.* In this graph plant extract shows percentage inhibition in all three solvent but ethanolic extract present better activity at all concentration.

Graph.3: Reducing power activity of plant extract in ethanol, aqueous and acetone solvent system



The graph shows *In-vitro* concentration dependent absorbance which showing reducing power activity by ethanolic, aqueous and acetone extract of *Actiniopteris dichotoma Bedd*. In this graph plant extract shows absorbance in all three solvent but ethanolic extract present better absorbance at all concentration.

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