ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Evaluation of Anti-Cancer and Anti-Oxidant Activity of *Cissus quandrangularis* Extracts in DMBA induced Mammary Carcinomas

Hemalatha C N[1], Anbarasu K[2], Vijey Aanandhi M[1*]

[1]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Vels University (VISTAS), Chennai, India

[2]Department of Pharmacology, KMCH College of Pharmacy, Coimbatore. India

*Corresponding author Email: **hodpchemistry@velsuniv.ac.in**

ABSTRACT:

Cancer is one of the major diseases and challenging to medicinal system to produce potent and the site specific anti-cancer drugs. In this cancer studies up to now most of the synthetic drugs produce undesirable side effects. At the same time from plant source produce potent and safer anti-cancer drugs than compare to synthetic drugs. *Cissus quadrangularis* is an important medicinal plant belonging to the family Vitaceae. In this study, the qualitative phytochemical studies by using ethanol and chloroform extracts, Toxicological studies by Acute toxicities studies (LD_{50}), Pharmacological Evaluations of Anti-tumor activity and Biochemical parameters are estimated and Anti-Oxidant Assay of *C.quadrangularis* were evaluated. The secondary metabolites such as phenol, glycosides, tannins and flavonoids were present in the ethanolic extract of *C.quadrangularis*. The DMBA breast cancer model is a well-known animal carcinogenesis model. *CQ* extracts possesses anti-tumor property as evidenced by its significant inhibition in the formation of tumor induced by chemical carcinogen (DMBA). From the results , it can be concluded that chloroform & ethanolic extract of *CQ* restored the biochemical imbalance occurred due to DMBA administration as well as lowered the tumor incidences and burden, the results obtained with the ethanolic extract were promising as compared to that of chloroform extract. Thus it may be useful for the treatment of mammary carcinoma and associated complications such as oxidative damage.

KEYWORDS: *Cissus quadrangularis* (CQ), Breast Cancer, 7, 12-dimethylbenz[α]anthracene (DMBA), Anti-cancer, In-vitro, Phytochemistry.

INTRODUCTION:

Cancer is a collective term for malignant neoplasm's arising in the body cells amounting to no less than hundred different disease states, each with its intrinsic natural history, etiology, methods of diagnosis and treatment. A tumor can be benign (not dangerous to health) or malignant (has the potential to be dangerous). Benign tumors are not considered cancerous: their cells are close to normal in appearance, they grow slowly, and they do not invade nearby tissues or spread to other parts of the body. Malignant tumors are cancerous. Left unchecked, malignant cells eventually can spread beyond the original tumor to other parts of the body.(1)

Breast cancer:

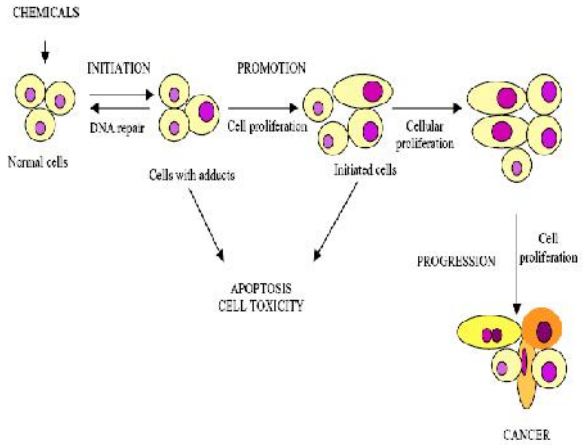
Breast cancer is an uncontrolled growth of breast cells. The term "breast cancer" refers to a malignant tumor that has developed from cells in the breast. Usually breast cancer either begins in the cells of the lobules, which are the milk-producing glands, or the ducts, the passages that drain milk from the lobules to the nipple. Less

Received on 21.08.2016 Modified on 28.11.2016
Accepted on 16.12.2016
Research J. Pharm. and Tech. 2017; 10(1): 293-300.
DOI: 10.5958/0974-360X.2017.00060.9

commonly, breast cancer can begin in the stromal tissues, which include the fatty and fibrous connective tissues of the breast.

Carcinogen:
Any substance that is capable of causing cancer is called as carcinogens. Carcinogens are chronic toxins. They cause damage after repeated or long-duration exposure. They may have not immediate apparent harmful effects, with cancer developing only after a long latency period.

***Chemical carcinogenesis stages*[2]**



MATERIALS AND METHODS:

Collection of Plant: Plant were locally collected and authenticated by Botanical survey of India (BSI)[3][4], Southern circle, Coimbatore, Tamilnadu.

Extraction procedure:
The plant *Cissus quadrangularis* Linn, were locally collected in month of June- July. The plant were chopped, air dried for a week and powdered. The powder was extracted in petroleum ether to remove fatty material using sox let apparatus[2]. Then the material was air dried and again subjected to extraction with chloroform till exhaustion. Then the material was air dried and again subjected to extraction with ethanol. The obtained extract was concentrated to remove excess of ethanol.

Animals:
Female Sprague-dawley rats weighing around 200g each were used.

Chemicals:
DMBA[5][6] was purchased from Sigma Chemical Company. Olive oil was purchased from local stores.

Phytochemical Analysis:
Qualitative Phytochemical[7][8] screening extracts of *C.quadrangularis* were screened for different phytochemical constituents' viz., carbohydrates, phenol, alkaloid, tannin, flavonoid and saponin. Phytochemical screening of the extracts was carried out by the standard methods. The results are tabulated in Table 2.

Chromatographic evaluation of ethanolic extract of *Cissus quadrangularis*:
Thin Layer Chromatography:
To support preliminary chemical analysis, ethanolic extract was subjected to TLC studies.

A) Separation of components:
The extract was dissolved in ethanol solvents separately and spotted using a calibrated capillary tube on a prepared TLC plate 1cm above from the bottom of the plate. The spot were equally sized and had a diameter ranging from 2-3 mm. The plant extracts subjected for separation i.e. ethanolic extract of *Cissus quadrangularis*. Results are shown in table number 3.

1) For the flavonoid component:
The solvent systems were used for separation is given below:
a. Chloroform: ethyl acetate (60:40)
b. Ethyl acetate: formic acid: glacial acetic acid 15%: ethyl methyl ketone: water (50:7:3:30:10)
c. Ethyl acetate: formic acid: glacial acetic acid 15%: water (100:11:11:26)
From the above solvent system, solvent no. 2 showed the good separation.

2) For the Alkaloid component:
Toluene: ethyl acetate: Di-ethyl amine (7:2:1)

3) For the glycoside component:
Ethyl acetate: ethanol: water (81:11:8)

4) For the sterols component: Chloroform

Determination of total phenols and flavanoids:
The Phenol content in the raw material of *Cissus quadrangularis* extract was estimated spectroscopically. Gallic acid was prepared as standard for phenols. The mean of three readings was used and the total phenolic content was expressed as milligrams of Gallic acid equivalents per gram extract and the results are tabulated in Table 4. Flavones and flavonoids in the ethanolic extracts of *Cissus quadrangularis* were estimated as Quercetin equivalent. Quercetin was used to make the calibration curve. The mean of three readings was used and the total flavonoids content was expressed in milligrams of Quercetin equivalents per gram extract and the results are tabulated in Table 5.

***In vitro* anti-oxidant activity:[9]**
DPPH Free Radical scavenging activity:
The DPPH Free Radical scavenging activity of *Cissus quadrangularis* extract was measured in terms of

hydrogen donating or radical scavenging ability using the stable radical DPPH (Blois, 1958). 0.1Mm solution of DPPH in ethanol was prepared and 1.0ml of this solution was added to 3.0ml of extract solution and standard in water at different concentrations (10-100µg/ml). 30 min later absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.[3]

$$\text{DPPH Scavenged (\%)} = \frac{A_{conc}-A_{test}}{A_{conc}} \times 100$$

Where A_{conc} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. The anti-oxidant activity of the extract was expressed as Ic50. The Ic50 value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

PHARMACOLOGICAL EVALUATION:

Toxicological studies:

Acute toxicity Studies:

Healthy adult Wistar albino mice of either sex, starved overnight were divided into five groups (n=6) and were orally fed with the Chloroform and Ethanolic extracts of *Cissus quadrangularis* in increasing dose levels of 100, 500, 1000, 2000 mg/kg body weight respectively. The animals were observed continuously for 2 hr. under the following profiles; Behavioral Profile; Alertness, restlessness, irritability, and fearfulness, Neurological profile; Spontaneous, Activity, reactivity, touch response, pain response and gait, Autonomic profile; Defecation and urination. After a period of 24 and 72 hr. they were observed for any lethality.

Animal study:

Female Sprague-Dawley rats at the age of seven weeks was purchased from Govt. veterinary College, Mannuthi, Thrissur, Kerala and maintain 12 hr. dark and 12 hr. light cycle and provide diet with water at *ad libitum.* In this preventive oriented study, the treatment was started on 0 day and induction of DMBA on 20 th day and treatment continued up to 60 days i.e., day 80. Female Sprague-dawley rats weighing around 200g each were divided into five groups, each comprising six animals:

GROUP I : Control,
GROUP II : DMBA (50mg/kg),
GROUP III : DMBA with $CHCl_3$ extract
GROUP IV : DMBA with Ethanolic extract,
GROUP V : DMBA with LETROZOLE

After completion of 20 days, prophylaxis treatment with plant extracts the group II, III, IV and V animals were induced the carcinogen (DMBA) at a dose of 50mg/kg. After carcinogenic induction the treatment was continued another 60days (i.e. up to day 80).

Preparation of DMBA solution:

DMBA was purchased from Sigma Chemicals, Mumbai, India. In this 240mg of DMBA was dissolved in 24ml of Olive oil. Each ml of Olive oil contains 10mg of DMBA.

DMBA induction in to Female Sprague-dawley rats:

DMBA was given to orally by using rat oral feeding needle. 1 week after the induction animals are subjected to palpitation to found the tumors.

Preparation of Letrozole solution:

Letrozole[(10)] is an oral non-steroidal aromatase inhibitor for the treatment of hormonally-responsive breast cancer after surgery.[4] Estrogens are produced by the conversion of androgens through the activity of the aromatase enzyme. Estrogens then bind to an estrogen receptor, which causes cells to divide. Letrozole prevents the aromatase from producing estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit. The Letrozole at a dose of 100µg was dissolved in 10 ml of distilled water as a vehicle. After DMBA administration, treatment with Letrozole was begun and continued for 60 days. Letrozole was given via orally at a dose of 10 µg/animal.

Preparations of *Cissus quadrangularis* extracts: T
he extracts were dissolved in Propylene Glycol, as a vehicle.

Estimation of haematological parameters[(11)]

After completion of treatment blood was collected and evaluated for WBC, RBC, Hb.

Estimation of haemoglobin:

Sahli's acid haematin method

Procedure:

By using pipette add 0.1 N HCl in the haemoglobinometer up to the lowest marking. Drop blood up to 20µl in the sahli's pipette. Adjusted the blood column carefully without bubbles. Wiped the excess of blood on the sides of the pipette by using a dry piece of cotton. Blown the blood into the acid solution in the graduated tube, rinsed the pipette well. Mixed the reaction and allow the mixture to stand at room temperature of 10 minutes. Diluted the solution with distilled water by adding few drops of water carefully and by mixing the reaction mixture until the colour matches the colour in the comparator. The lower meniscus of the fluid was noted and reading was noted in g/100ml.

Estimation of total R.B.C count:
Procedure: Blood was taken up to 0.5 mark, in the R.B.C pipette and diluting fluid up to 101 mark. It was mixed well and kept for 3 minutes. 2-3 drops of fluid was discarded, from the pipette, which did not contain any cells. The counting chamber was charged after 3 minutes (to allow the cells to settle). The R.B.C in the square were marked 'R' in figure under high power. R.B.C count was then calculated.

Estimation of total W.B.C count:
Procedure: The W.B.C pipette was cleaned, dried and blood was sucked up to 0.5 mark, then immediately the diluting fluid was sucked up to the mark 11. Mixing was done by rolling the pipette horizontally in the palms. [5-7] First 2 to 3 drops of the mixture was discarded, then charged the chamber with dilatant. Air bubble should be avoided, focused the field under low power and calculated the total number of W.B.C. in 64 squares. The estimation results of hematological parameters are tabulated in **Table 6.**

Biochemical parameters:
Serum glutamate oxalo acetate transaminase (SGOT):
Method:
Optimized UV- test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).
Reagents:
Reagent 1: TRIS Ph 7.8 (80 mmol/l), L- Aspartate (240 mmol/l), MDH(malatedehydrogenase) ≥600 U/l, LDH(lactate dehydrogenase) ≥600 U/l.
Reagent 2: 2-Oxaloglutarate(12 mmol/l), NADH (0.18 mmol), Pyridoxal-5-Phosphate FS, Good buffer pH (9.6 0.7 mmol/l),Pyridoxal-5-Phosphate (0.09 mmol/l).

Assay procedure:
Mixed 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube. To this, added 100 µl of serum. Mixed well and took the reading immediately. Normal range: < 41 u/l

Serum glutamate pyruvate transaminase (SGPT)[(12)]:
Method:
Kinetic UV test, according to the international federation of clinical chemistry and laboratory medicine (IFCC).

Reagents:
R1: TRIS PH 7.5 (100 mmol/l) L-Alanine (500 mmol/l),, LDH (lactate dehydrogenase) ≥1200 U/l,
R2: 2-Oxoglutarate(15mmol/l), NADH (0.18 mmol/l), Pyridoxal -5-Phosphate FS , Good's buffer PH 9.6 (0.7mmol/l), Pyridoxal -5-Phosphate (0.09 mmol/l)

Assay procedure:
Mixed 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube. To this, added 100 µl of serum. Mixed well and took the reading immediately. Normal range: < 41 u/l

Estimation of urea:
Estimation of urea was did by Urease-GLDH: enzymatic UV test.

Reagents:
Reagent 1: TRIS PH 7.8 (120mmol/l), **2**-Oxoglutarate (7mmol/l), ADP(0.6 mmol/l)**,** Urease(≥ 6 KU/l)**,** GLDH(≥ 1 KU/l)
Reagent 2: NADH (0.25 mmol/l)
Reagent 3: Standard (40 mg/dl)

Procedure:
1000 µl of reagent-1 and 250 µl of reagent- 2 were taken in a 5 ml test tube and 10 µl serum was added. Mixed well and immediately the test sample was read at 340 nm Hg 334 nm, Hg 365 nm optical path 1 cm against reagent blank (2-point kinetic).

Estimation of creatinine:
Estimation of Creatinine by Jaffe Method (modified)
Reagents:
Reagent 1 Standard Creatinine- 2mg/100ml
Reagent 2 Picric acid solution.,
Reagent 3 sodium hydroxide solution .

Procedure:
500 µl of reagent -2 and 500 µl of reagent -3 was taken in a 5ml test tube and 100 µl of serum was added. Mixed well and immediately the test sample was read at Hg 492 nm 1cm light path. The results of biochemical parameters are tabulated in **Table 7.**

Estimation of protein and aminoacids:
Reagents Solution A:
1ml $CuSO_4$ $5H_2O$ (1%) + 1ml sodium potassium tartrate (2%) + 98 ml 2% Na_2CO_3 in 0.1N NaOH. Solution B: Folin Ciocalteu reagent and distilled water mixed in 1:1 ratio just before use.

Procedure:
0.01 ml of tissue homogenate (2.5%) was diluted to 1.2 ml and mixed with 6 ml of solution A. The mixture was incubated at room temperature for 10 min and adds 0.3 ml solution B was added, mixed immediately and kept at room temperature for 30 min. Optical density was taken at 750 nm. The amount of protein was calculated from the standard curve of bovine serum albumin (BSA). The results are tabulated in **Table 8.**

IN-VIVO ANTI-OXIDANT ACTIVITY:
Enzymatic Anti-oxidant activity[(13)]: Estimation of catalase activity[(14)](Table 9), Estimation of Superoxide dismutase (SOD)[(15)] Activity: (Table 10) and Estimation of Glutathione peroxidase (GPx) [(16)]Activity: (Table 11)

have been done and results are tabulated.

RESULTS:

Preliminary tests:

Table 1. Physical characteristics of extracts of CQ

S.No	NAME OF EXTRACT	COLOUR
1	**CQ**-Chloroform extract	**Dark Brownish**
2	**CQ**-Ethanolic ectract	**Yellowish**

Table 2. Qualitative chemical tests of Chloroform and Ethanolic extract of *Cissus quadrangularis*

S. No	Phytochemical constituents	*CQ*-$CHCL_3$ extract	*CQ*-ETHANOLIC extract
1.	Alkaloids	-	-
2.	Carbohydrates	-	-
3.	Flavanoids	+	+
4.	Glycosides	+	+
5.	Protein and Amino acids	+	+
6.	Saponins	+	+
7.	Steroids and Sterols	-	+
8.	Triterpenoids	-	+

Thin layer chromatography:

Table 3: Shows TLC pattern of ethanolic extract of *Cissus quadrangularis*

S.No	Plant extract	No. Of Spots	Rf value
			0.18
1	Plant extract	3	0.41
			0.82
2	Quercitin	1	0.85

Dertermination of total phenols

Table 4: Shows total Phenols of ethanolic extract of *Cissus quadrangularis.*

S.No	Sample	Concentration (µg/ml)	Absorbance
1	Gallic acid	10	0.262
		20	0.314
		30	0.413
		40	0.533
2	CQ extract	20	0.107

The total phenolic compound in ethanolic extract of *Cissus quadrangularis* extract was found to be **67 mg/100 g** calculated as gallic acid equivalent.

DETERMINATION OF TOTAL FLAVONOIDS

Table 5: Total flavonoids content of Ethanolic extract of *Cissus quadrangularis*

S.No	Sample	Concentration (µg/ml)	Absorbance
1	Quercetin	20	0.238
		40	0.350
		60	0.408
		80	0.476
		100	0.557
2	CQ extract	10	0.153

Total Flavanoids compound in Ethanolic extract of *Cissus quadrangularis* was found to be **10.57 mg/100g** calculated as Quercetin equivalent.

PHARMACOLOGICAL EVALUATION:

Evaluation of Mammary Tumor Activity: Estimation of Hematological Parameters

Table 6: Shows the effect of Ethanolic extract of *Cissus quadrangularis* on the level RBC, WBC and Hb counts of the control and experimental rats

Group(n=6)	Normal	DMBA only	DMBA+CQ-CHL extract(100mg/kg)	DMBA+CQ-ET extract(100mg/kg)	DMBA+LET (10µg/kg)
WBC	5.12±1.2	13.25±0.19[a]	8.26±0.11[a]	8.01±0.18[a]	8.10±0.15[a]
RBC	14.29±0.35	11.16±0.65[a]	12.54±0.28[a]	13.59±0.74[a]	13.01±0.09[a]
Hb	13.27±0.42	8.14±0.61[a]	11.35±0.55[a]	12.85±0.48[a]	12.57±0.09[a]

a-P<0.001, b-P<0.01, c-P<0.05, ns-nonsignificant Data was expressed as mean ±SEM (n=6 animals in each group). Values were statistically extremely significant at P<0.001

BIOCHEMICAL PARAMETERS:

Table 7: Shows the effect of ethanolic extract of *Cissus quadrangularis* on the level of SGPT, SGOT, Creatinine ,Urea in the liver of the control and experimental rats

Group (n=6)	Normal	DMBAonly	DMBA+CQ-CHL extract(100mg/kg)	DMBA+CQ-ET extract(100mg/kg)	DMBA+LET (10µg/kg)
SGPT	78.66± 0.91	140.33±1.83[a]	97.0±2.28[a]	70.0±0.73[a]	70.33± 1.52[a]
SGOT	131.0± 9.25	213.0±3.34[a]	202.66±3.83[ns]	139.66±4.23[a]	125.66± 5.18[a]
creatinine	0.63± 0.02	0.73±0.02[b]	0.73±0.02[ns]	0.7±0.02[ns]	0.66± 0.02[ns]
Urea	38.56± 0.18	47.5±1.87[c]	30.93±1.84[a]	28.4±1.99[a]	29.73± 2.60[a]

a-P< 0.001,b-P<0.01,c-P<0.05, ns-non significant. Data was expressed as mean ±SD. (n=6 animals in each group). Values were statistically extremely significant at P<0.001. unit: unit/mg of protein

ESTIMATION OF PROTEIN

Table 8: Shows the effect of ethanolic extract of *Cissus quadrangularis* on the level of total protein in liver and kidney of the control and experimental rats.

Group (n=6)	Normal	DMBA only	DMBA+CQ-CHL extract(100mg/kg)	DMBA+CQ-ET extract(100mg/kg)	DMBA+LET (10µg/kg)
Liver	0.087± 0.001	0.089±0.00^{ns}	0.373±0.176 ^{ns}	0.100±0.001 ^{ns}	0.094±0.00 ^{ns}
Kidney	0.087± 0.001	0.082± 0.00^{b}	0.079±0.001 ^{a}	0.077±0.00 ^{a}	0.076±0.00^{a}

a-P< 0.001,b-P<0.01,c-P<0.05, ns-non significant

Data was expressed as mean ±SD.(n=6 animals in each group). Values were statistically extremely significant at P<0.001. unit: unit/mg of protein.

ANTI-OXIDANT ACTIVITY:

In Vivo Anti- oxidant Activity - Enzymatic Antioxidant Activity: Estimation Of Catalase Activity

Table 9: Shows the effect of Ethanolic extract of *Cissus quadrangularis* on levels of catalase in liver and kidney of control and experimental rats

Group (n=6)	Normal	DMBA only	DMBA+CQ-CHL extract(100mg/kg)	DMBA+CQ-ET extract(100mg/kg)	DMBA+LET (10µg/kg)
Liver	317.49± 28.57	235.30± 11.22^{b}	403.36 ± 13.54 ^{a}	279.98±5.04^{c}	363.63± 9.15^{a}
Kidney	279.13± 15.32	189.40± 15.55 ^{a}	503.01±11.23 ^{a}	554.56±24.90 ^{a}	827.02±9.34 ^{a}

a-P< 0.001,b-P<0.01,c-P<0.05 ns-non significant

Data was expressed as mean ±SD.(n=6 animals in each group). Values were statistically extremely significant at P<0.001. unit: unit/mg of protein

ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Table 10: Shows the effect of Ethanolic extract of *Cissus quadrangularis* on levels of SOD in liver and kidney of control and experimental rats

Group (n=6)	Normal	DMBA only	DMBA+CQ-CHL extract(100mg/kg)	DMBA+CQ-ET extract(100mg/kg)	DMBA+LET (10µg/kg)
Liver	0.0408± 0.0034	0.0041± 0.0009	0.0316 ± 0.0013^{ns}	0.0623 ± 0.0146 ^{a}	0.0638± 0.0029 ^{a}
Kidney	0.0366± 0.0024	0.0163± 0.0007	0.0302 ± 0.0013 ^{ns}	0.0502 ± 0.0128^{c}	0.0734± 0.0052 ^{a}

a-P< 0.001,b-P<0.01,c-P<0.05 ns-non significant

Data was expressed as mean ±SD. (n=6 animals in each group). Values were statistically extremely significant at P<0.001. unit: unit/mg of protein

ESTIMATION OF GLUTATHIONE PEROXIDASE (GPx) ACTIVITY:

Table 11: Shows the effect of Ethanolic extract of *Cissus quadrangularis* on levels of GPx in liver and kidney of control and experimental rats

Group (n=6)	Normal	DMBAonly	DMBA+CQ-CHL extract(100mg/kg)	DMBA+CQ-ET extract(100mg/kg)	DMBA+LET (10µg/kg)
Liver	206.04±11.79	97.41± 4.77 ^{a}	132.64±17.92 ^{ns}	238.44±6.45 ^{a}	230.94±3.37 ^{a}
Kidney	269.71±4.68	87.37± 5.35 ^{a}	227.23±16.32 ^{a}	262.33±6.45 ^{a}	291.59±2.45 ^{a}

a-P< 0.001,b-P<0.01,c-P<0.05 ns-non significant. Data was expressed as mean ±SD.(n=6 animals in each group). Values were statistically extremely significant at P<0.001. unit: unit/mg of protein

NON-ENZYMATIC ANTIOXIDANT ACTIVITY :

Estimation of reduced glutathione (GSH):

Table 12: Shows the effect of Ethanolic extract of *Cissus quadrangularis* on levels of GSH in liver and kidney of control and experimental rats

Group (n=6)	Normal	DMBAonly	DMBA+CQ-CHL extract(100mg/kg)	DMBA+CQ-ET extract(100mg/kg)	DMBA+LET (10µg/kg)
Liver	130.46± 8.59	65.83± 2.42 ^{a}	244.19±24.47 ^{a}	228.73±1.52 ^{a}	230.40 ± 13.61 ^{a}
Kidney	107.69± 1.20	60.07± 3.12 ^{a}	283.54±4.50 ^{a}	271.09±16.92 ^{a}	222.58 ± 11.59 ^{a}

a-P< 0.001,b-P<0.01,c-P<0.05 ns-non significant

Data was expressed as mean ± SEM. (n=6 animals in each group). Values were statistically extremely significant at P<0.001. unit: unit/mg of protein

In Vitro Anti-Oxidant Activity : DPPH Radical scavenging activity:

Table 13: Shows % DPPH radical scavenging activity of CQ extract

CQ extract	Concentration(µg/ml)				
	100	200	300	400	500
% Inhibition	45.21	56.52	55.79	68.03	74.95

Table 14: Shows % DPPH radical scavenging activity of Quercetin standards

Quercetin	Concentration(µg/ml)				
	10	20	30	40	50
% Inhibition	43.53	55.74	65.55	72.22	83.63

Role Of *Cissus quadrangularis* On The Mammary Carcinogenesis

S.NO	GROUPS	LATENCY	TUMOR BURDEN
1	DMBA only	4.5±0.25	3±0.25
2	DMBA+CQ-CHL extract(100mg/kg)	5.5±0.45	2±0.50
3	DMBA+CQ-ET extract(100mg/kg)	7±0.50	1±0.75
4	DMBA+LET (10µg/kg)	12±0.50	1±0.25

a-P< 0.001,b-P<0.01,c-P<0.05 ns-non significant. Data was expressed as mean ± SEM. (n=6 animals in each group). Values were statistically extremely significant at P<0.001.

TUMOR INCIDENCES

Table 15: shows the effect of *Cissus quadrangularis* on tumor incidences (%)

S.NO	GROUPS	NO.OF RATS WITH TUMOR	INCIDENCES (%)
1	DMBA only	6/6	100
2	DMBA+CQ-CHL extract(100mg/kg)	4/6	66
3	DMBA+CQ-ET extract(100mg/kg)	2/6	33
4	DMBA+LET(10µg/kg)	2/6	33

DISCUSSION:

Chemoprevention has the potential of providing an important means for cancer prevention, for both the general population and even more importantly for individuals at high risk. So it is becoming increasingly importantly to screen natural products which might suppress or reverse the process of carcinogenesis. The DMBA breast cancer model is a well-known animal carcinogenesis model. Several proposed mechanisms have been implicated by investigators. One is that DMBA is metabolized to generate reactive oxidative stress (ROS) to induce severe damage or adducts on the biomolecules. Numerous anti-oxidants have shown to inhibit both the initiating and the promoting phase of DMBA induced rat mammary carcinogenesis. The present study provides ample evidence that *CQ* could inhibits the DMBA induced up-regulation of aryl-hydrocarbon receptor and over production of proliferating cell nuclear antigen (PCNA) that ultimately reduced the tumor formation in rats. The results of this study demonstrate that *CQ* extracts possesses anti-tumor property as evidenced by its significant inhibition in the formation of tumor induced by chemical carcinogen (DMBA). The plant extracts were tested for the presence of various phytochemical constituents, which were reported to have distinctive medicinal properties. The acute toxicity tests on mice showed that up to 2gm/kg *CQ* extract was non toxic. In order to establish the relationship between the chemical content and the anti-tumor activity, the total phenol and total flavonoid contents of ethanolic extract of CQ were determined. The result obtained from experiment revealed that the ethanolic extract of CQ contains higher concentration of total phenols (67mg/g) of extract calculated as Gallic acid equivalent and flavonoid (10.57mg/g) of extract calculated as Rutin equivalent. These findings suggest that there can be a correlation between the higher concentration of total phenols and total flavonoids content and anti-tumor activity.

The DPPH radicals was absorbed at 515nm, this absorption is inhibited in the presence of anti-oxidants. This reduction in absorbance is related to the antiradical efficiency of the extract. Table 13 shown that DPPH antiradical efficiency value of the ethanolic extract of CQ. Superoxide dismutase (SOD) a pair of superoxide anions by oxidizing one to oxygen and other to hydrogen peroxide. Hydrogen peroxide which also be mutagenic, is degraded to H_2O and oxygen by catalase. The decrease in the level of catalase and sod in DMBA only rats and subsequent increase in the treated groups. Table 9 and 10 shown that administration of cq extract significantly increases the level of CAT and SOD. Reflects the anticarcinogenic potential and ability to revert the decreased levels of CAT and SOD to normalcy. Glutathione peroxidase (GPx) has been shown to be an important adaptive response to condition of increased peroxidative stress. Table 11 represents increase in GPx level in cq extract treated animals as compared with the DMBA treated animals in a dose-dependent manner. GSH is a tripeptide with a reactive sulfhydryl group; this plays a central role in the co-coordinating the body's anti-oxidant defence process. GSH is the first line of defence against peroxidant status. This thiol group participates in the protection against deleterious effects of reactive oxygen species evolved during biological imbalance as well as cancerous conditions. In this study, observed a decrease in the GSH levels in DMBA induced rats, this represents increased utilization due to oxidative stress. Table 12 shows the administration of cq extract increase the content of GSH in the liver of the treated rats.

The results obtained in this study can be confirmed by the fact that the *CQ* extract also showed significant changes in altered biochemical parameters caused by the carcinogen treatment in strengthening of the antioxidant system. The restoration of normal *in vivo* antioxidant system, and results obtained from the *invitro* anti-oxidant studies as well as the effects of mammary tumor genesis

and body weight analysis, are suggestive of the potential mammary carcinoma protective activity of *CQ* against the DMBA induced mammary carcinoma and provide further rationale for the potential development of this traditional medicinal plant as an effective chemo preventive agent against the mammary carcinomas.

ACKNOWLEDGEMENT:

The authors are thankful to Vels University (VISTAS) and its management for providing research facilities and encouragement.

REFERNCES:

1. Sen R.Principles and management of CANCER, A practical Guide; 2004:1-5
2. Oliveira PA, Aura Colaco, Raquel Chaves. Chemical carcinogenesis. *An Acad Bras Cienc* 2007:79(4); 593-616.
3. Bishayee A, Oinam S, Basu M, Chatterjee M, Vanadium. Chemoprevention of 7, 12-dimethylbenz(*a*)anthracene-induced rat mammary carcinogenesis: probable involvement of representative hepatic phase I and II xenobiotic metabolizing enzymes, *Breast Cancer Res. Treat*.2000:63(2); 133–145
4. Sugiyama T, Sakan M, Ueda N. DMBA induced rat leukemia. *Leukemia Res* 2002: 26; 1053:1068.
5. Robert GW, Wang Qing-feng, Wang Yong, You-Hao. A Taxonomic Investigation of Variation Within Cissus quadrangularis L.(Vitaceae) In Kenya College of Life Sciences, Wuhan University, *J of nat.sci* ,2001:6(3).
6. Natarajan B, Paulsen BS *and* Pushpangadan P . An Ethnopharmacological Study from the Coimbatore District, Tamil Nadu, India: Traditional Knowledge Compared With Modern Biological Science. *Pharmaceutical Biology*, 1999:37(5); 378-390.
7. Sen SP. Study of active constituents (ketosteroids) of CQ. *Indian J of pharmacol*. 1964:4; 247.
8. Pluemaj T and E. Saifah, Constituents of Cissus quadrangularis Linn, *J Pharm Sci* 1986:11;205–211. .
9. Jainu M, Devi CS. In vitro and in vivo evaluation of free radical scavenging potential of cissus quqdrangularis. *African J of biomed Res.* 2005:8; 95-99..
10. Haynes BP, Dowsett M, Miller WR, Dixon JM. The pharmacology of letrozole. *J of steroid biochem and molec biol* 2003:87; 35-45.
11. Udayakumar, R., M. Sundaran and Raghuram Krishna. Mineral and biochemical analysis of various parts of Cissus quadrangularis Linn. *Ancient Science of Life*, 2004:26(2); 20-23.
12. Berger MG, Sprengart ML, Kusnan M, Fock HP. Ammonia Fixation via Glutamine Synthetase and Glutamate Synthase in the CAM Plant Cissus quadrangularis L. 1986:81(2); 356-360.
13. Chidambara Murthy KN, A. Vanitha, M. Mahadeva Swamy, G.A. Ravishankar. Antioxidant and Antimicrobial Activity of *Cissus quadrangularis* L *Journal of Medicinal Food.* 2003:6(2); 99-105
14. Sinha et.al, conducted a study on Estimation of catalase activity in 1972.
15. Kakkar et.al, conducted a study on Estimation of Superoxide dismutase (SOD) activity in 1984.
16. Rotruck.et.al, Conducted a study on Estimation of Glutathione peroxidase (GPx) Activity in 1973.