World Journal of Science and Research 1(1): 12-25 (2016).

ISSN: 2455 2208

Available online at http://www.harmanpublications.com

World Journal of Science and Research

Research Article Botany

ANTICANCER ACTIVITY OF *Clerodendrum phlomidis* (L) LEAVES AND ITS NANOPARTICLES

V. Lakshmi[1] and G. Viji Stella Bai[2]

[1]Research Scholar, Department of Botany, Kundavai Naachiyaar Government Arts College For Women (Autonomous), Thanjavur, Tamil Nadu, S. India
[2]Department of Botany, Kundavai Naachiyaar Government Arts College for Women (Autonomous), Thanjavur, Tamil Nadu, S. India

ABSTRACT

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year. In the present study to investigate the anticancer activity of *Clerodendrum phlomidis* (L) leaves extract (CPLE) and its nanoparticles. It is evident that silver nanoparticles (SNPs) and CPLE is capable of modulating the enzymatic and non enzymatic antioxidant defense mechanisms and restored the tumor and diagnostic markers in Diethylnitrosamine (DEN) induced hepatocellular carcinogenesis. The morphometric and histological studies also supported the chemopreventive properties of SNPs and CPLE and all these observations clearly indicate a significant antitumor activity of SNPs and CPLE. Our study confirms that SNPs and CPLE plays duel role by blocking carcinogen metabolic activation and enhancing carcinogen detoxification. Protective properties of the CPLE may be due to the presence of phytochemicals such as flavonoids, terpenoids alkaloids etc. SNPs possess potential anticancer activity than CPLE due to nano size particles act efficiently than crud extract.

Citation: Lakshmi V and G. Viji Stella Bai (2016). Anticancer activity of *Clerodendrum phlomidis* (L) leaves and its nanoparticles. World Journal of Science and Research. 1(1): 12-25.

Article Info:

Received on 29 January 2016
Accepted on 15 Feb. 2016

Keywords:
Cancer, *Clerodendrum phlomidis*, Tumour markers, Oxidative stress

Corresponding author
Dr. G. Viji Stella Bai
Professor,
Department of Botany,
Kundavai Naachiyaar Government Arts College for Women (Autonomous),
Thanjavur, Tamil Nadu, S. India

INTRODUCTION

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year. It is the second major cause of deaths after cardiovascular diseases. Cancer is a general term applied of series of malignant diseases that may affect different parts of body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. These cells are born due to imbalance in the body and by correcting this imbalance the cancer may be treated (Siegel and Zhu, 2009).

Diethylnitrosamine is an N-nitroso alkyl compound, categorized as a potent hepatotoxin and hepatocarcinogen in experimental animals, producing reproducible tumors after repeated administration. The main cause for concern is that diethylnitrosamine is found in a wide variety of foods like cheese, soybean, smoked, salted and dried fish, cured meat and alcoholic beverages (Liao *et al.*, 2001). Metabolism of certain therapeutic drugs is also reported to produce diethylnitrosamine (Akintonwa, 1985). It is also found in tobacco smoke at a concentration ranging from 1 to 28 ng/cigarette and in baby bottle nipples at a level of 10 ppb (IARC, 1972). Diethylnitrosamine is reported to undergo metabolic activation by cytochrome P_{450} enzymes to form reactive electrophiles which cause

oxidative stress leading to cytotoxicity, mutagenicity and carcinogenicity (Janani *et al.*, 2010). The detection of diethylnitrosamine in commonly consumed food products makes the human population vulnerable to its exposure. This constraint underscores the need for the development of novel hepatoprotective drug with potent antioxidant activity.

Various plants and plant derived products have been tested and found to be effective against diethylnitrosamine induced hepatocarcinogenesis and hepatotoxicity (Jayakumar *et al.*, 2012). Use of plants for treating various ailments of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. (Uniyal *et al.*,2003). In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Ayurveda, Siddha and Unani) (Dahanukar *et al.*, 2000). The medicinal value of the chosen plant *Clerodendrum phlomidis* leaves has not been extensively worked out. Therefore, the present study was to examine the anticancer activity of *Clerodendrum phlomidis* leaves and its silver nanoparticle.

MATERIALS AND METHODS

Preparation of plant powder

The *Clerodendrum phlomidis* leaves were collected and dried under shade. These dried leaves were mechanically powdered and stored in an airtight container. These powdered materials were used for further analysis.

Preparation of alcoholic extract

The powder *C. phlomidis* leaf was extracted with 70% ethanol for 48 hours.A semi solid extract was obtained after complete elimination of alcohol under reduced pressure.The *C. phlomidis* L. leaf extract (CPLE) was stored in refrigerator until used.

Synthesis of silver nanoparticles

For the Ag nanoparticles synthesis, 5 ml of *Clerodendrum phlomidis* is leaf extract was added to 45 ml of 1 mM and 4 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark at 5 hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaf extract. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the silver nanoparticles (AgNPs) were freeze dried using SEM analysis (Arunachalam *et al*., 2012).

PHARMACOLOGICAL STUDIES

Animals

Male albino rats of Wistar strain approximately weighing 180-200g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and watter were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fibre (4.02%), Ash (8.02%) and sand silical (1.02%).

Chemicals

Diethylnitosamine, Nitro blue tetrazolium (NBT), ethylene diamine tetraacetic acid (EDTA), trichloro acetic acid (TCA), thiobarbituric acid (TBA), 1-chloro-2,4-dintiro benzene (CDNB), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized), streptozotocin (STZ), Diethylnitrosamine (DEN) and L-ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Plant material and preparation of extract

The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37°C. The dried materials was ground into make a fine powder and used for extraction. Three hundred grams (300g) of the leaves powder plants were extracted with ethanol (70%) using "Soxhlet Apparatus" for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract contains both polar and non-polar phytocomponents. For experiments 500mg/kg body weight of *Clerodendrum phlomidis* leaf extract (CPLE) was used. This effective dose was selected based on dose dependent studies of CPLE carried out in our laboratory.

Experimental design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows. Group 1: Normal control rats fed with standard diet and pure drinking water. Group 2: Rats induced with hepatocellular carcinoma by providing 0.01% DEN through drinking water for 16 weeks. Group 3: Rats pretreated with Nanoparticles (10mg/kg) intragastrically at the dose of (500 mg/kg body weight) for one week before the administration of

DEN and continued till the end of the experiment (i.e., 16 weeks). Group 4: Rats treated with *Clerodendrum phlomidis* L. leaves extract alone by oral gavage daily at a dose of 500 mg/kg body weight (based on effective dosage fixation studies) for 16 weeks.

Collection of blood and preparation of serum sample

At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood was collected by cardiac puncture into serum separator tubes. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000rpm for 10minutes, and then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

Tissue homogenate

Immediately after blood collecting, the animals were sacrificed by cervical dislocation and the liver was dissected out, washed with ice-cold physiological saline. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris Hcl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

Histological studies

The liver tissue was fixed in 10% normal saline for 72 h after which the tissues were sliced to a thickness of 2.1mm each. These were dehydrated using alcohol of graded concentration. They were further treated with paraffin wax and cast into blocks; sections of the tissues were cut on a microtome to 5 µm. These were later attached to a slide and dried. The samples slides were viewed on a photographic microscope to find out histological changes.

BIOCHEMICAL ESTIMATIONS

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Superoxide dismutase activity was determined by the procedure of Kakkar *et al.* (1984) in plasma. The activity of catalase was assayed by the method of Beers and Sizer (1952). The activity of glutathione peroxidase was assayed by the method of Rotruck *et al* (1973). Reduced glutathione was estimated by method of Moron *et al* (1979). The level of ascorbic acid was estimated by the method of Omaye *et al* (1979). α-tocopherol was estimated by the method of Baker *et al* (1980).Protein was estimated by the method of Lowry *et al.*,. (1951). The serum SGOT and SGPT were estimated by the method of Reitman and Frankel (1957). The tumor marker alpha-fetoprotein was measured by radioimmunoassay techniques (Eiken Chemical Co., Japan). Serum homocysteine levels were estimated by ELISA method of Prlmus *et al.,* (1988). Serum α2M was determined using radial-immuno diffusion kit (Biocientifica SA, Buenos Aires-Argentina). CEA was measured in serum by chemiluminescent immunoassay (Fully Automated ADVIA Centaur, Bayer, USA). DNA was estimated by the method of Burton (1956). RNA was estimated by the method of Rawal *et al* (1977).

RESULTS

Anticancer activity of *Clerodendrum phlomidis* leaves extract and its Silver nanoparticles

Evaluation of lipid peroxidation (LPO)

Table 1 shows the level of LPO in the liver of control and experimental groups of animals which was analyzed for oxidative stress. In DEN-induced group 2 animals, there is a significant increase in the levels of lipid peroxides and protein carbonyl when compared with group 1 normal control animals. This could be a tumor burden. Whereas in CPLE - treated group 3 animals, there is a significant decrease in the levels of LPO when compared with group 2 tumor-bearing animals. However, animals treated with silver nanoparticles (SNPs) (group 4) did not show any significant changes when compared with group 1 control animals.

Evaluation of antioxidant status in liver

Table 1 depicts the antioxidant status in the liver of control and experimental group of animals. DEN-induced group 2 animals exhibited a significant decrease in the activities of SOD and CAT when compared with group 1 normal control animals, *Clerodendrum phlomidis* leaves extract (CPLE) treated group 3 showed a significant increase in the activities of SOD and CAT when compared with group 2 DEN-induced animals. The activities of GPx and GSH also significantly decreased in DEN induced group 3 tumor-bearing animals when compared with group 1 control animals. In SNPs -treated group 4 animals, there is a significant increase in the activities of GPx and GSH when compared with group 2 DEN-induced animals.

The levels of antioxidant vitamins, vitamin-C and vitamin-E were also decreased in rats treated with diethylnitrosamine as compared to control (Table 2). CPLE and SNPs treatment for 16 weeks caused a significant reversal of the fall in the levels of vitamin-C and vitamin-E in the liver tissue. In SNPs -treated group 4 animals, there is a significant increased in the vitamin-C and vitamin-E when compared with group 2 DEN-induced animals.

Evaluation of liver tumour markers

The content of the tumour markers (serum AFP, α2M, Hcy, CA and liver DNA and RNA in control and experimental animals were found to be a significant alterations in tumor bearing animals when compared with control (Table 3). The rise ($p<0.05$) in the content of AFP, α2M, Hcy and Cancer antigen found in group 2 tumor bearing

animals was significantly decreased in group 3 CPLE treated animals when compared with tumor bearing group. In SNPs -treated group 4 animals, there is a significant restored the tumour markers when compared with group 2 DEN-induced animals.

Tumor bearing animals showed a significantly increased nucleic acid contents (DNA and RNA) in liver tissues (Table 3). CPLE (500 mg/kg) treatment resulted in a significant decrease in the levels of nucleic acid contents in group 3 animals as compared with group 2 rats. In SNPs -treated group 4 animals, there is a significant restored the DNA and RNA when compared with group 2 DEN-induced animals.

The activity of AST and ALT in the serum of control and experimental animals is presented in Table 4. It is observed that administration of DEN (Group-III) to rats produced a significant increase in the activities of all the marker enzymes, the increase being two-fold for AST and ALT, when compared to control rats. In SNPs -treated group 4 animals, there is a significant decreased in the activity of AST and ALT when compared with group 2 DEN-induced animals. Treatment with CPLE administration (Group-3) significantly decreased the activities of these enzymes to normalcy.

Table 1 Effect of *Clerodendrum phlomidis* leaves and nanoparticle on liver MDA, SOD, Catalase and GPx activities in experimental rats

Parameters	Group I	Group II	Group III	Group IV
MDA (nmole/gm tissues)	2.87 ±0.20[a]	3.86±0.27[b]	2.32±0.16[a]	2.97±0.20[a]
SOD (U/ mg protein in tissues)	5.58 ±0.23[a]	3.98 ±0.19[b]	4.92 ±0.27[a]	5.32 ±0.24[a]
Catalase (U/ mg protein in tissues)	4.72 ± 0.23[a]	3.51 ±0.20[b]	5.78 ±0.28[a]	4.80 ±0.24[a]
GPx (U/ mg protein in tissues)	6.21 ±0.43[a]	4.72 ± 0.33[b]	7.63 ±0.38[a]	6.73 ± 0.33[a]

Values are expressed as Mean ± SD for six rats

Values which are not sharing common superscript differ significantly at 5% level (P<0.05). Duncan Multiple Range Test (DMRT).

Table 2 Effect of *Clerodendrum phlomidis* leaves and nanoparticle on liver GSH, Vitamin C and Vitamin E content in experimental rats

Parameters	Group I	Group II	Group III	Group IV
GSH (µg/mg tissues)	6.21 ± 0.49[a]	4.56 ± 0.32[b]	7.63 ± 0.60[a]	6.73± 0.50[a]
Vitamin C (µg/mg tissues)	9.32 ±0.45[a]	6.45 ±0.50[b]	11.34 ± 0.56[a]	10.03 ± 0.50[a]
Vitamin E (µg/mg tissues)	6.53 ±0.24[a]	4.23 ±0.25[b]	6.45 ±0.32[a]	6.54 ±0.26[a]

Values are expressed as Mean ± SD for six rats

Values which are not sharing common superscript differ significantly at 5% level (P<0.05). Duncan Multiple Range Test (DMRT).

Table 3 Effect of *Clerodendrum phlomidis* leaves and nanoparticle on tumour markers in experimental rats

Parameters	Group I	Group II	Group III	Group IV
AFP (Units)	0.5 ±0.04 [a]	0.97 ± 0.07 [b]	0.43 ± 0.03 [a]	0.57 ± 0.05 [a]
HCY (µmole/l)	4.3 ± 0.27 [a]	9.5 ± 0.29 [b]	5.7 ± 0.18 [a]	5.2 ±0.15 [a]
CEA (Units)	0.35 ±0.003 [a]	0.49 ± 0.004 [b]	0.36 ± 0.15 [a]	0.38 ± 0.009 [a]
α2M ( )	131.8± 4.2 [a]	223.8±5.4 [b]	133.17± 3.99 [a]	136.7±3.0 [a]
DNA (mg/g tissue)	1.78 ± 0.04 [a]	2.5 ± 0.14 [b]	1.86 ± 0.08 [a]	1.75 ±0.13 [a]
RNA (mg/g tissue)	1.4 ± 0.09 [a]	2.8 ± 0.07 [b]	1.4 ± 0.2 [a]	1.6 ± 0.08 [a]

Values are expressed as Mean ± SD for six rats

Values which are not sharing common superscript differ significantly at 5% level (P<0.05).Duncan Multiple Range Test (DMRT).

Table 4 Effect of *Clerodendrum phlomidis* leaves and nanoparticle on diagnostic markers in experimental rats

Parameters	Group I	Group II	Group III	Group IV
Protein (gm/dl)	6.83 ± 0.27 [a]	4.49 ±0.35 [b]	6.06 ± 0.28 [a]	6.63 ± 0.14 [a]
AST (IU/L)	60.24 ± 5.42 [a]	82.58 ± 6.63 [b]	64.83±532 [a]	64.34 ± 5.79 [a]
ALT (IU/L)	36.08 ± 3.08 [a]	65.05 ± 4.11[b]	37.36 ± 3.67 [a]	37.04 ± 3.33 [a]

Values are expressed as Mean ± SD for six rats

Values which are not sharing common superscript differ significantly at 5% level (P<0.05). Duncan Multiple Range Test (DMRT).

Morphometric analysis

Macroscopic appearance of the liver cells of control group 1 animals shows normal morphology (Plate 1a). DEN alone administered group 2 animals showing enlargement and several grayish white nodules and foci on the peripheral surface of the liver (Plate 1b). Liver cell shows normal morphology in compound alone treated group 3 animals (Plate 1c). Most of the foci and nodules disappeared in the liver from DEN and SNPs treated group (group 4) of rats showing the effect of chemoprevention (Plate 1d).

Histopathological observation

Histological examination of the liver showed normal architecture in both normal control group (Plate 2a) and CPLE supplemented rats (Plate 2c). However, cellular damage with malignancy was obvious in the DEN treated liver. The liver showed loss of architecture, neoplastic hepatocytes with large cells, vesicular nuclei and prominent nucleoli. It showed nodular arrangement (Pseudo lobule formation) surrounded by lymphocytic infiltrate (Plate 2b). In contrast, DEN with SNPs co-treatment showed near normal hepatocytes with lymphocytic infiltration formed around the central vein without disruption of the liver architecture (Plate 2d).

DISCUSSION

Synthesis of silver nanoparticle

Silver nanoparticles are being extensively synthesized using many different biological sources including fungi, bacteria and plants (Shivaji *et al.*, 2011; Shaligram *et al.*, 2009). Among them the plant mediated nanoparticles synthesis is getting more popular because of the high reactivity of plant extract and easy availability of plant materials. This method of nanoparticles synthesis involves no toxic chemicals and termed as green chemistry procedure. In this present study, *Clerodendrum phlomidis* L. extract was used for the synthesis of silver nanoparticles. The aqueous $AgNO_3$ solution turned to brown colour with the addition of leaf extract, indicating the formation of AgNPs in the

Plate 1 Morphometric study of Clerodendrum phlomidis L. leaf and Nanoparticle treated rats

Plate 1a-Control

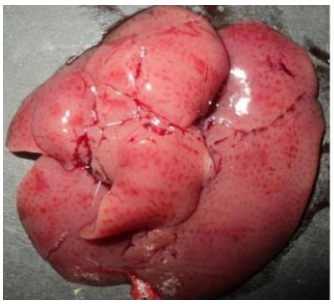


Plate 1b- DEN induced cancer rat

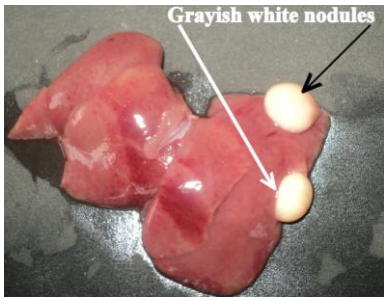


Plate 3c DEN + Nanoparticle treated

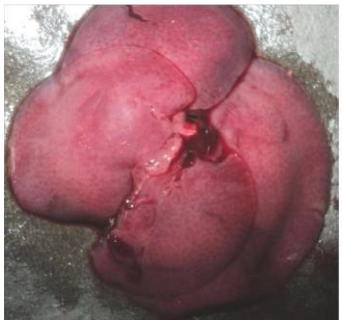


Plate 4d DEN + *Clerodendrum phlomidis* treated

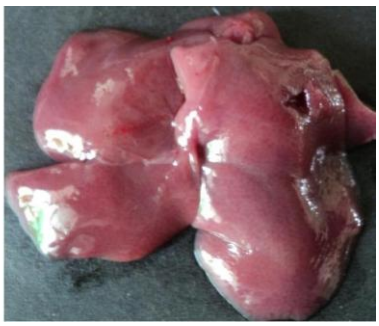


Plate 2: Histological examination of the liver experimental rats

Plate 2a Control

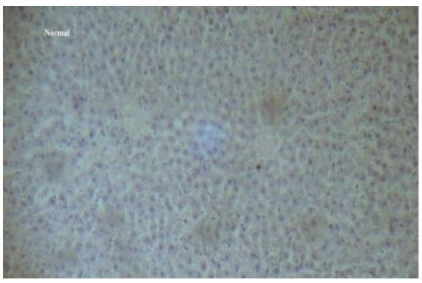


Plate 2b DEN induced cancer rat

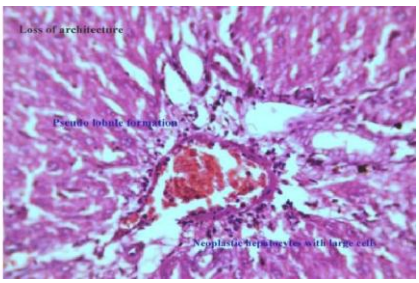


Plate 2c DEN + Nanoparticle treated

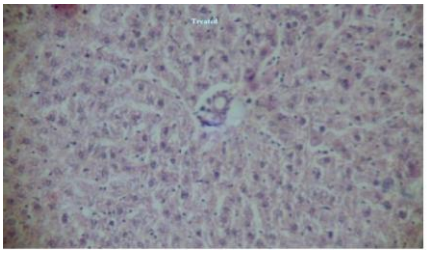
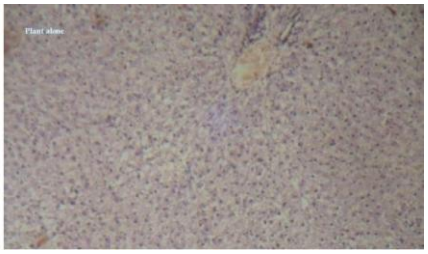


Plate 2d DEN + *Clerodendrum phlomidis* L. treated



reaction solution probably as a result of the excitation of surface plasmon resonance (SPR) bands (Mulvaney, 1996). The control ($AgNO_3$) showed no change in colour when incubated in a similar condition.

There was a visible colour change after the substrate was provided to the plant extract. Initially the plant extract was light green. Upon providing the silver salt, it turned red. The presence of nanoparticles was confirmed by obtaining a spectrum in visible range of 200nm to 300nm. A typical peak at 438nm was obtained due to the surface plasmon resonance of silver nanoparticles. The ethanolic extract also showed a colour change from light green to brown. Further, upon subjecting to the spectrum in visible range, a peak at 415nm was obtained showing presence of silver nanoparticles.

SEM analysis was carried out to understand the topology and the size of the Ag-NPs, which showed the synthesis of higher density polydispersed spherical Ag-NPs of various sizes. The SEM image showing the high density silver nanoparticle synthesized by the leaf extract further confirmed the development of silver nanostructures. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size between 98.85 to 344.23 nm as well the cubic, face-centred cubic structure of the nanoparticles.

Thirumurugan *et al*., (2010) reported the extracellular biological synthesis of silver nanoparticles (AgNP`s) using plant leaf extracts (*Lantana camara)* for the reduction of aqueous Ag+ ions. Stable silver nanoparticles were formed by treating aqueous solution of $AgNO_3$ with the plant leaf extracts as reducing agent of Ag^+ to Ag^0. The formation of yellowish brown colour, confirmed the synthesized silver nanoparticles and UV-visible spectroscopy (430nm) was used to monitor the quantitative formation of silver nanoparticles. The size and shape (40nm) of the nanoparticles was characterized by SEM (Scanning Electron Microscopy). In this presence study the formation of silver nanoparticales with *C.phlomidis* L. is succussfully carried out.

Anticancer activity

Green synthesis of silver nanoparticles (SNPs) using green resources like *Clerodendrum phlomidis* L. leaves is a better alternative to chemical synthesis, since this green synthesis has many advantages such as, ease of applicability for large scale production, economically feasible, and ecofriendly. The anticancer studies showed great improvement in the biological activity of the extracts after SNPs synthesis. It is well known that Ag ions and Ag-based compounds have strong biological activities (Furno *et al*., 2004). Owing to their small size, SNPs impair the sulfur and phosphorus containing essential macromolecules such as proteins and DNA (Wei *et al*., 2009), thus, action of SNPs appears to be a consequence of adherence to and penetration inside the cell of the target cells. The anti-proliferative effect of SNPs and Ag was reported (Ahamed *et al*., 2008; Rahman *et al*., 2009).

Zolghadri and co-workers demonstrated that silver nanoparticles (SNPs) provide a relatively high hydrophobicity inside bovine hemoglobin which causes a transition from alpha helixes to beta sheets and leads to partial unfolding and aggregation of the protein (Zolghadri *et al*., 2009), other researchers suggest that SNPs are likely to interact with thiol rich enzymes (Morones *et al*., 2005); Therefore, it is possible that once penetrated into cells, SNPs may attack functional proteins of cells which results in partial unfolding and aggregation of proteins as it is the case in the bovine hemoglobin. Toxicity of silver nanoparticles is concentration-size-shape dependent ; In green synthesis process these factors are affected by chemical compositions of plant parts extracts, accordingly this will lead to variability in the biological activities of such extracts (Shawkey *et al*., 2013).

Tumorigenesis is a multistep process that begins with cellular transformation and progresses to hyper proliferation leading to metastatic lesions. This progress can be activated by the carcinogenotoxic substances which are widely employed to develop cancers in specific organs of experimental animals. DEN is a potent carcinogen for HCC. DEN is well known to cause perturbations in the nuclear enzymes involved in DNA repair and is normally used as a carcinogen to induce liver cancer in animal models and these compounds are considered to be effective health hazards in causing HCC (Hahn and Weinberg, 2002).

Liver plays a pivotal role in regulation of physiological processes such as metabolism, secretion and storage. Unfortunately it is a common target for a number of toxicants. The multitude of pathological changes caused by the progression of tumor as well as its inhibition through chemotherapy is expected to be reflected in the biochemical and histological

parameters of the host system, particularly pertaining to the liver which is known to be the major organ affected in carcinogenesis (Mayer and Kulkarni, 2001).

Oxidative stress is associated with damage to a wide range of macromolecular species including lipids, proteins, and nucleic acids thereby producing major interrelated derangements of cellular metabolism including peroxidation of lipids. Free radicals and non-radicals oxidizing Species were produced in animals treated with carcinogens, and also in human tissues (Sun, 1990). Reactive oxygen species (ROS) is formed from endogenous or exogenous sources are highly reactive, toxic, and mutagenic (Halliwell, 1994). DEN has been shown to generate free radicals (Halliwell and Gutteridge, 1989), an uncompromising free radical generation in the liver overwhelms the antioxidant status and ultimately proceeds to oxidative stress paving way to carcinogenesis (Gey, 1993). Lipid peroxidation plays an important role in carcinogenesis (Banakar *et al.,* 2004), is the most studied biologically relevant free radical chain reaction and measured as malonaldehyde (MDA). Induction of DEN has been reported to generate lipid peroxidation products like malondialdehyde and 4-hydroxy nonenal that may interact with various molecules leading to cause oxidative stress and carcinogenicity (Hietanen *et al.*, 1987).

Increased level of LPO was reported during DEN-induced hepato carcinogenesis. This dynamic action may further lead to uncompromised production of free radicals overwhelming the cellular antioxidant defense (Klaunig and Kamendulis, 2004). Our result agrees with the earlier reports (Aml *et al.,* 2010; Dhanasekaran *et al.,* 2009). It has been extensively reported that free radicals participated in DEN-induced hepatocarcinogenesis. MDA generation at the initiation stage can be prevented by free radicals scavengers and antioxidant action of CPLE. Animals treated with SNPs and CPLE exhibited significantly lowered the levels of LPO in liver when compared with animals induced with DEN. This shows the anti-lipid peroxidative and anti-protein oxidation role of SNPs and CPLE that is probably mediated by its ability to scavenge free radical generation.

Antioxidants possess a variety of biological activities, including the induction of drug-metabolizing enzymes, inhibition of prostaglandin synthesis, inhibition of carcinogen- induced mutagenesis, and scavenging of free radicals (Hirose *et al.*, 1994). Antioxidants may protect membrane from ROS toxicity by prevention of ROS formation by the interruption of ROS attack, by facilitating the repair caused by ROS and by providing cofactors for the effective functioning of other antioxidants (Sen, 1995). Development of life threatening diseases like cancer is linked to the availability of these antioxidants (Gutteridge, 1994). Natural antioxidants are capable of inhibiting the ROS production and thereby reducing the associated intracellular oxidative stress (Feng *et al.,* 2001).

SOD is the first line of defense in the antioxidant system against the oxidative damage mediated by superoxide radicals (Oberley and Oberley, 1986). Superoxide dismutases catalyze the dismutation of superoxide radical to hydrogen peroxide and water (Mccord and Fridovich, 1969). Furthermore, CAT or GPx catalyze the transformation of H_2O_2 to harmless byproducts. Glutathione, a cysteine-containing tripeptide, is required to maintain the normal reduced state of cells and to counteract all the deleterious effects of oxidative stress. GSH is said to be involved in many cellular processes including the detoxification of endogenous and exogenous compounds. DEN, an electrophilic carcinogen may interact with the large nucleophilic pool of GSH thereby reducing the macromolecule and carcinogen interaction (Chasseaud, 1979). In SNPs and CPLE treated animals, there was a significantly higher level of GSH in liver when compared to DEN-induced animals consistent with the idea of attenuation of DNA carcinogen interaction and thereby averting a favorable environment for carcinogenesis.

Decreases in the activities of SOD, CAT and GPx are seen in tumor cells. The compounds that can scavenge excessive free radicals in the body are suggested to hinder the process of carcinogenesis (Sumathi *et al.*, 1996). Such studies support our findings as we had seen a significant decrease in the activities of antioxidant enzyme in liver of animals treated with carcinogen in comparison with normal animals. Reduction in antioxidant enzyme (SOD, CAT, GPx) activities in liver by DEN is consistent with the earlier reports (Aml *et al.,* 2010; Dhanasekaran *et al.,* 2009). On the other hand, there is a significant increase in the activities of antioxidant enzymes in liver of the animals administered SNPs and CPLE when compared with animals administered carcinogen alone.

Excessive liver damage and oxidative stress caused by diethylnitrosamine depleted the levels of non-enzymic antioxidants like GSH, vitamin-C and vitamin-E in our study.

Non-enzymic antioxidants like vitamin-C and E act synergistically to scavenge the free radicals formed in the biological system. GSH acts synergistically with vitamin-E in inhibiting oxidative stress and acts against lipid peroxidation (Chaudiere, 1994). Vitamin-C also scavenges and detoxifies free radicals in combination with vitamin-E and glutathione (George, 2003). It plays a vital role by regenerating the reduced form of vitamin-E and preventing the formation of excessive free radicals (Das, 1994). The decreased levels of these antioxidant vitamins and GSH observed during diethylnitrosamine administration might be due to the excessive utilization of these vitamins in scavenging the free radicals formed during the metabolism of diethylnitrosamine. SNPs and CPLE treatment effectively restored the depleted levels of these nonenzymic antioxidants caused by diethylnitrosamine. Increase in GSH levels in the present study observed that turn contributes to the recycling of other antioxidants such as vitamin-E and vitamin-C (Exner *et al*., 2000). This shows that SNPs and CPLE maintain the levels of antioxidant vitamins by maintaining GSH homeostasis, thereby protecting the cells from further oxidative stress.

The positive modulation of cellular damage in liver induced by the chronic feeding of carcinogens was also evident through the electron microscopic studies, like SEM of liver, the target organ. There were clear evidences of the SNPs and CPLE providing protective action to liver. The histopathological studies also supported the protective action of SNPs and CPLE.

It is well known that liver cancer is one of the most important cancers in the world, resulting in more than 1 million patients and over 260,000 deaths per year (Liu *et al* ., 2006). Therefore, the chemoprevention and treatment of liver cancer is very important. AFP, α2M, Hcy, DNA, RNA CEA and liver weight are valuable references, widely used in animal studies to diagnose and observe the development of hepatocarcinogenesis (Thirunavukkarasu *et al* ., 2005). In the present study, the values of previously mentioned parameters showed sharp alterations in DEN-group as compared with that of the normal control group.

Terpenoids exert antiproliferative and antitumour effects that are particularly pronounced in tumour cells (Lage *et al.*, 2010). Terpenoids are believed to be active against cancer by enzymatically promoting glutathione transferase (Fahey and Sundquit, 1991). Our study showed significant decrease in body weight and normalization of liver weight caused in SNPs and CPLE-group. The decrease in body weight in SNPs and CPLE-supplemented group may be attributed at least partly to the slowing down of digestion by inhibiting enzymes such as amylase, protease and lipase activities at levels, which could affect carbohydrates, protein, and fat digestion and absorption. Our findings are in concordance with McDougall and Stewart (2005) study.

AFP, a tumour-associated fetal protein, has long been employed as a serum fetal tumour marker to monitor disease progression (Abelev, 1971; Liu *et al* ., 2006). The observed significant increase of serum α2-macroglobulin in DEN-induced rats is in harmony with Sukata *et al*., (2004) who stated that α2M might be tightly linked to the rat hepatocarcinogenesis from the initial stage to tumour progression even in conditions, which are undetectable, by established cytochemical markers such as placental glutathione-S-transferase (GST-P) and γ-GT-positive lesions. Sukata *et al.*,(2004) also confirmed that the observed increases in serum α2M concentrations during hepatocarcinogenesis and in animals, bearing hepatic tumours was not a result of secretion by the host liver of α2M as an acute-phase reactant in response to inflamematory injury. α2M functions as a carrier protein and re regulator for various growth factors and cytokines such as transforming growth factor-β (known to be involved in the onset of hepatocyte apoptosis) (James, 1990). Furthermore, α2M partially counteracts the inhibitory effects of transforming growth factor-β on proliferation of neoplastic hepatocytes, suggesting that under some conditions, α2M can promote hepatocarcinogenesis by perturbing transforming growth factor-β-induced apoptosis (Wollenberg *et al* ., 1991). In the present study observed that the increased content of AFP, CEA and α2M concentrations in cancer bearing animals. Supplementation of SNPs and CPLE to cancer bearing animals restored the content of AFP, CEA and α2M concentrations. Our findings are in concordance with Nermin *et al.,* (2008) study, which have been reported that supplementations of SNPs and CPLE decrease the content of AFP and α2M concentrations on DEN induced liver cancer.

Nucleic acid content of tumor is found to be an important indicator of prognosis, because it is well correlated with the size of the tumor in the cancerous condition (Gallagher, 1986). In diseased state, the degree of malignancy increases with the

defective abnormalities in DNA. Reports reveal that abnormal amount of DNA was observed in various cancers including breast carcinoma, endometrial carcinoma and lung carcinoma (Ellis *et al.*, 1991). In the present study, an increased activity was observed in DEN induced liver cancer animals and this may be due to the over expression of many enzymes which are necessary for DNA synthesis in tumor cells.

RNA levels were found to be increased in the cancerous condition as DNA and RNA are directly related to each other, an abnormally increased content of DNA may lead to an increased transcription, which in turn increased RNA content in tumor cells. The mechanisms by which tea polyphenols may act includes the inhibition of promutagen activation, the inactivation of mutagens and carcinogens, blocking and scavenging of reactive molecules, modulation of DNA replication or repair, inhibition of promotion, and inhibition of invasion and metastasis of tumor cells. These mechanisms are currently being progressively clarified. Most of the reports on mechanisms, however, still remain as suggestive or speculative (Kuroda and Hara, 1999). Present findings are similar to the Pakkir *et al.*, (2011) study. In SNPs and CPLE treated animals, the nucleic acid levels were decreased due to its inhibition of mutagenesis process.

Increased serum Hcy activities seem to be interrelated in the present study. Animal and human studies have increasingly demonstrated associations between folate deficiency, serum Hcy elevations, and a variety of cancers. The observed increase in serum Hcy in our study is suggested to reflect inhibition of homocysteine metabolism due to folate deficiency reported in other studies (Eichholzer *et al* ., 2001; Davis and Uthus, 2004). Folate is important for normal DNA synthesis, repair, and converting homocysteine to methionine (Davis and Uthus, 2004). Therefore, increased demand of folate is postulated to be a result of increased hepatic levels of DNA and RNA and might indicate increased DNA and RNA synthesis and proliferation of cancer cells in response to growth stimulation.

Based on the present study, the action model of SNPs may be described as SNPs making a breakthrough in the permeability of outer membrane firstly, resulting in the leakage of cellular materials. Secondly, SNPs enter the inner membrane and inactivate respiratory chain dehydrogenases, thus inhibiting respiration and growth of cells. Simultaneously, SNPs could affect some proteins and phosphate lipids and induce collapse of membrane, resulting in cell decomposition and death eventually (Shawkey *et al*., 2013).

These results are potentially promising because they suggest that, by using non-cytotoxic amounts of silver salt with a convenient, eco-friendly and cheap method using *Clerodendrum phlomidis* L aqueous extract; we can synthesize SNPs with good anticancer activities. This opens the door to prepare a suitable pharmaceutical formulation using these nanoparticles.

In conclusion, it is evident that SNPs and CPLE is capable of modulating the enzymatic and non enzymatic antioxidant defense mechanisms and restored the tumor and diagnostic markers in DEN-induced hepatocellular carcinogenesis. The histological studies also supported the chemopreventive properties of SNPs and CPLE and all these observations clearly indicate a significant antitumor activity of SNPs and CPLE, further evidenced by macroscopic appearance of the liver. Our study confirms that SNPs and CPLE plays duel role by blocking carcinogen metabolic activation and enhancing carcinogen detoxification. Protective properties of the CPLE may be due to the presence of phytochemicals such as flavonoids, terpenoids alkaloids etc. SNPs possess potential anticancer activity than CPLE. Thus, a study of the exact mechanism by which SNPs inhibit signalling cascades responsible for the development and progression of the disease would be a tremendous breakthrough in the field of nanomedicines and make these agents an effective alternative in tumor and angiogenesis-related diseases.

REFERENCES

Siegel AB and Zhu AX. (2009) Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. *Cancer*, 115: 5651–61.

Ellis, CN, Burnette CN, Sedlack R, Dyas C and Balkemore WS. (1991) Prognostic applications of DNA analysis in solid malignant lesions in humans. 173: 329-42.

Shawkey AM, Mohamed A. Rabeh, Abeer K, Abdulall and Ashraf O, Abdellatif. (2013) Green nanotechnology: Anticancer Activity of Silver Nanoparticles using *Citrullus colocynthis* aqueous extracts.

Advances in Life Science and Technology. 13: 60-72.

Davis CD and Uthus EO (2004). DNA methylation, cancer susceptibility, and nutrient interactions. *Exp. Biol. Med*. 229(10): 988-995.

Kuroda Y and Hara Y. (1999) Antimutagenic and anticarcinogenic activity of tea polyphenols. *Mutation Res*. 436: 69-97.

Eichholzer M, Lüthy J, Moser U and Fowler B. (2001) Folate and the risk of colorectal, breast and cervix cancer: the epidemiological evidence. Swiss Med. Wkly. 131(37-38): 539-549.

Reitman S and Frankel S. (1957) A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am J Clin Path* : 25: 56.

Gallagher RE. (1986) Biochemistry of neoplasia. In: Comprehensive textbook of oncology. Moosa AR, Robson MC, Schimpff SC (eds). Baltimore, USA, *Williams and Wilkins*, 36-45.

Wollenberg GK, LaMarre J, Semple E, Farber E, Gauldie J and Hayes MA. (1991) Counteracting effects of dexamethasone and alpha 2-macroglobulin on inhibition of proliferation of normal and neoplastic rat hepatocytes by transforming growth factors-beta type 1 and type 2. *Int. J. Cancer*, 47:311–316.

Sukata T, Uwagawa S, Ozaki K, Sumida K, Kikuchi K and Kushida M. (2004) alpha(2)-Macroglobulin: a novel cytochemical marker characterizing preneoplastic and neoplastic rat liver lesions negative for hitherto established cytochemical markers. *Am. J. Pathol*. 165(5):1479-1488.

Nermin AH, Sadik, Shohda A, EL-Maraghy, Manal F and Ismail. (2008) Diethylnitrosamine-induced hepatocarcinogenesis in Rats: possible chemoprevention by blueberries. *African Journal of Biochemistry Research*, 2 (3), 081-087.

McDougall GJ and Stewart D. (2005) The inhibitory effects of berry polyphenols on digestive enzymes. *Biofactors*. 23(4), 189-195.

Liu JG, Zhao HJ, Liu YJ and Wang XL. (2006) Effect of selenium-enriched malt on hepatocarcinogenesis, paraneoplastic syndrome and the hormones regulating blood glucose in rats treated by diethylnitrosamine. *Life Sci.* 78(20), 2315-2321.

James K. (1990) Interactions between cytokines and _2-macroglobulin. *Immunol*. Today. 11,163–166.

Fahey, R.C., Sundquist, A.R.,(1991). Evolution of glutathione metabolism. Adv Enzmol **64**,1–53

Thirunavukkarasu C, Premkumar K, Jagadeeswaran R, SakthisekaranD (2005). The inhibitory effect of sodium selenite on Nnitrosodiethylamine-induced and phenobarbital promoted liver tumourigenesis in rats based on the modulation of polyamine levels. Mol. Cell Biochem. 280(1-2):165-172.

Abelev, G.I., (1971). Alpha - fetoprotein in association with malignant tumors. *Adv. Cancer Res.***14,** 295–357.

Liu H, Liu X, Lee J, Liu Y, Yang H, Wang G, *et al*. (2008) Insulin therapy restores impaired function and expression of P-glycoprotein in blood–brain barrier of experimental diabetes. Biochemical Pharma-cology;75:1649–58.

Exner, R., Wessner, B., Manhart, N., Roth, E., (2000). Therapeutic potential of glutathione. Wien. Klin. Wochenschr. **112**, 610–616.

Dhanasekaran, M., Baskar, A.A., Ignacimuthu, S., Agastian, Duraipandiya, V., (2009). Chemopreventive potential of Epoxy clerodane diterpene from *Tinospora cordifolia* against diethylnitrosamine-induced hepatocellular carcinoma. Invest New Drugs. **27,**347–355

Debajyoti Das.(2015).GC-MS analysis of bioactive compounds in the methanol extract of Clerodendrum viscosum leaves.Pharmacognosy Res 2015 Jan-Mar;7(1):110-3.

Aml, FM., Ibrahima, H.M.S., Shalaby, M.A., (2010). Protective Effect of Broccoli and Red Cabbage Against Hepatocellular Carcinoma Induced by N- Nitrosodiethylamine in Rats. *Journal of American Science*, **6(12)**, 1136-45.

Sen CK (1995) Oxygen toxicity and antioxidants: state of the art. Indian J Physiol Pharmacol 39:177–196

Gutteridge, J.M., (1994). Antioxidants, nutritional supplement and life threatening diseases. Br J Biomed Sci **51,**288–295

Sumathi R, Baskaran G, Varalakshmi P (1996) Effect of DL-lipoic acid on tissue redox state in acute cadmium-challenged tissues. J Nutr Biochem 7:85–92.

Chaudiere, J., (1994). Some chemical and biochemical constrains of oxidative stress in living cells. In: Rice-Evans, C.A., Burdon, R.H. (Eds.), Free radical damage and its control. Elsevier Science, Amsterdam. 25–66.

George VC1, Kumar DR, Rajkumar V, Suresh PK, Kumar RA.(2012). Quantitative assessment of the relative antineoplastic potential of the n-butanolic leaf extract of Annona muricata Linn. in normal and immortalized human cell lines. Asian Pac J Cancer Prev. ;13(2):699-704.

Klaunig, J.E., Kamendulis, L.M., (2004). The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol **44,**239–267.

Mccord, J.M., Fridovich, I., (1969). The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide and oxygen. *J Biol Chem***244,**6056–6063

Chasseaud, L.F., (1979). The role of glutathione and glutathione-stransferase in the metabolism of chemical carcinogens and other electrophilic agents. Adv Cancer Res **29,**175–274

Sun Y (1990) Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med 8:583–599.

Halliwell ,B., Gutteridge, J.M.C., (1989). Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity.In: Cheeseman KH (ed) Free radicals in biology and medicine. Clarendon Press, Oxford, 144–147

Burton K., 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemical Journal 62,315-323.

Banakar, M.C., Paramasivan, S.K., Chattopadhyay, M.B., Datta, S., Chakraborty, P., Chatterjee, M., Kannan, K., Thyagarajan, E., (2004). 1a, 25-dihydroxyvitamin D3 prevents DNA damage and restores antioxidant enzymes in rat hepatocarcinogenesis induced by diethylnitrosamine and promoted by phenobarbitol. World J Gastroenterol **10(9),**1268–1275

Hirose, M., Imaida, K., Tamano, S., (1994). Cancer chemoprevention by antioxidants. In: Ho CT (ed) Food phytochemicals: teas, spices and herbs. American Chemical Society Press, Washington, 122–132

Mayer, S.A., Kulkarni, A.P., (2001). Hepatotoxicity. In: Hodgson E, Smart RC (eds) Introduction to biochemical toxicology. *Wiley, New York*, 599–628.

Gey, K.F., (1993). Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. Br Med Bull **49,**679–699.

Hietanen, E., Ahotupa, M., Bartsch, H., (1987). Lipid peroxidation and chemically induced cancer in rats fed lipid rich diet. In: Lapis K (ed) In carcinogensis and tumor progression. Akademiaikiado Press, Budapest, 9–16

Hahn ,W.C., Weinberg, R.A., (2002). Rules for making human tumor cells. N Engl J Med **347**,1593–1603.

Feng, Q., Kumagai, T., Torii, Y., Nakamura, Y., Osawa, T., Uchida, K., (2001). Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress. Free Radic Res **35,**779–788.

Halliwell, B. (1994). Free radicals, antioxidant and human disease: curiosity, cause or consequence? Lancet 344,721–724.

Shivaji S, Madhu S, Shashi S. (2011) Extracellular synthesis of antibacterial silver nanoparticles using Psychrophilic bacteria. Process Biochem;46:1800–7.

Shaligram NS, Bule M, Bhambure R, Singhal RS, Singh SK, Szakacs G, Pandey A. (2009) Biosynthesis of silver nanoparticles using aqueous extract from the compactin producing fungal strain. Process Biochem;44:939–43.

Thirumurugan A., Neethu Anns Tomy, Hema Priyanka Kumar, Prakash.P Biological synthesis of silver nanoparticles by *Lantana camara* leaf extracts.International Journal of Nanomaterials and Biostructures 2011; 1 (2) 22-24

Zolghadri, S., Saboury, A., Golestani, A., Divsalar, A., Rezaei-Zarchi, S., Moosavi-Movahedi, A., 2009. Interaction between silver nanoparticle and bovine hemoglobin

at different temperatures. Journal of Nanoparticle Research 11, 1751-1758.

Furno, F., Morley, K.S., Wong, B., Sharp, B.L., Arnold, P.L., Howdle, S.M., Bayston, R., Brown, P.D., Winship, P.D., Reid, H.J., 2004. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? Journal of Antimicrobial Chemotherapy 54, 1019-1024.

Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., Yacaman, M.J., 2005. The bactericidal effect of silver nanoparticles. Nanotechnology 16, 2346.

Ahamed, M., Karns, M., Goodson, M., Rowe, J., Hussain, S.M., Schlager, J.J., Hong, Y., 2008. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicology and Applied Pharmacology 233, 404-410.

Wei, D., Sun, W., Qian, W., Ye, Y., Ma, X., 2009. The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. Carbohydrate research 344, 2375-2382.

Oberley LW, Oberley TD (1986) Free radicals, cancer and aging. In: Johnson J (ed) Free radicals, aging and degenerative diseases. Alan R Liss Inc, New York, pp 325–371.

Omaye ST., Tumball JD., Sauberlich HE. (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymology .62: pp1-11.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin's reagent. J Biol Chem. 1951; 193: 265-276.

Prlmus J, KeIIey EA, Hansen HJ, and Goldenberg DM. "Sandwich"-TypeImmunoassayof CarcinoembryonicAntigen in Patients Receiving Murine MonoclonalAntibodiesfor Diagnosisand Therapy Clin. Chem. 34/2, 261-264 (1988)

Rawal Um, Patel US Rao and Desai RR (1977) Clinical and biochemical studies on cateractous human lens III. Quantitative study of protein, RNA and DNA. Arogya J Health Science. 3: 69-72.

Mulvaney P. (1996). Surface plasmon spectroscopy of nanosized metal particles. Langmuir;12:788–800.

Baker H., Frank O., De Angeles B and Feinglod S. (1980) Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutrition Reports International 21: pp531.

Moron MS., DsePierre JW and Manerwik KB. (1979) Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochimica et Biophysica Acta 582: pp67-68.

Liao, D.J., Blanck, P., Eneroth, J.A., Gustafsson, I.P., Hallstrom(2001). Diethylnitrosamine causes pituitary damage, disturbs hormone levels, and reduces sexual dimorphism of certain liver functions in the rat, Environ. Health Perspect. **109**,943–947.

Akintonwa D.A., (1985).The derivation of nitrosamines from some therapeutic amines in the human environment, Ecotoxicol. Environ. **9**, 64–70.

IARC, Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Man, vol. 1, International Agency for Research on Cancer, Lyon, 1972.

Janani, P., Sivakumari, K., Geetha, A., Ravisankar, B., (2010). Chandrakesan Parthasarathy. Chemopreventive effect of bacoside A on *N*-nitrosodiethylamineinduced hepatocarcinogenesis in rats. J Cancer Res Clin Oncol **136,**759–770.

Jayakumar, S., Madankumar, A., Asokkumar, S., Raghunandhakumar, S., Gokula dhas, K., Kamaraj, S., Divya S.G.J., Devaki, T.,(2012). Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. Mol Cell Biochem **360**, 51–60.

Uniyal B, Utilization of medicinal plants by the rural women of Kulu, Himachal Pradesh. *Indian Journal of Traditional Knowledge.* **2(4):** pp. 366-370. (2003).

Dahanukar S A, Kulkarni R A and Rege N N; Pharmacology of medicinal plants and natural Products. Ind J Pharmacol, 2000; 32: 81-118.

Arunachalam R, Dhanasingh S, Kalimuthu B, Uthirappan M, Rose C, Asit Baran M. (2012) .Phytosynthesis of silver nanoparticles using Coccinia grandis

leaf extract and its application in the photocatalytic degradation. Colloids and Surfaces B: Biointerfaces 94 226– 230

Beuge JA and Aust SD. (1978) The thiobarbituric acid assay. Methods in Enzymology 52: pp 306-307.

Kakkar P., Das B and Viswanathan PN. (1984) A modified spectrophotometric assay of SOD. Indian Journal of Biochemistry and Biophysics 21: pp130-132.

Beers R and Sizer I. (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry 195: p133.

Rotruck JT., Pope AL., Ganther HE., Swanson AB., Hafeman DG and Hoekstra WG. (1973) Selenium: biochemical roles as component of glutathione peroxidase. Science. 179: pp588-590.