Therapeutic Potential of *Cucumis melo* (L.) Fruit Extract and Its Silver Nanoparticiles Against DEN-Induced Hepatocellular Cancer in Rats

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Abstract

Biosynthesized silver nanoparticles have a wide range of biological activities and using nanoparticles as one of the novel approaches in cancer therapy. In this present research work, the anti-cancer efficacy of *Cucumis melo* fruit extract and its silver nanoparticles was explored. Wistar rats were divided into six groups and hepatic cancer was induced with 0.01% DEN (diethylnitrosamine) through drinking water for 16 weeks. Cyclophosphamide was given as the standard drug at the dose of 50 mg/kg body weight. Hematological parameters showed a decrease in the levels of hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), and platelets (PLTS) levels except white blood cell (WBC) in DEN-induced cancer animals. Significant alterations in the hematological parameters were observed after treatment which indicate the protective effect of *Cucumis melo* fruit on the hemopoietic system. The structural integrity of the cells has been damaged in cancer-induced animals, and this results in cytoplasmic leakage of enzyme into the blood stream, leads to the elevated levels of these enzymes in blood with subsequent fall in the tissues. Hence, the levels of liver function markers such as AST ALT, ALP, LDH, GGT, and 5’NT were significantly elevated in serum and the liver of cancer-induced rats. The levels of serum tumor markers, viz., alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA), elevated in rats induced with DEN, which then were reduced following *Cucumis melo* fruit treatment, indicating the anti-cancer activity of the drug. Histological evaluation of the liver and kidney was also performed to authenticate the present work. Treatment with crude extract and silver nanoparticles of *Cucumis melo* fruit indicates that *Cucumis melo* fruit could have exerted its protective effect.

Keywords *Cucumis melo* (L) · Silver nanoparticles · Hematological profile · Liver function markers · Tumor markers

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Introduction

India is bestowed with the enormous biodiversity of medicinal plants. The World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their health needs due to better cultural acceptability, fewer side effects, and better compatibility with the human body [1]. Nanotechnology, an interdisciplinary research field comprising chemistry, engineering, biology, and medicine, has great potential for early detection, meticulous diagnosis, and tailored treatment of cancer. Medicinal plants have been widely used for the treatment of diseases in a traditional way for several years. Herbal nanoparticles can be utilized to increase the herbal drug solubility and help to localize the drug in a specific site thus resulting in better efficacy and improved patient compliance. In the last 20 years, a variety of nanoparticle-based therapeutic and diagnostic agents are developed for the treatment of cancer, diabetes, pain, asthma, allergy, and infections [2].

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and affects more than half a million people worldwide, with the highest incidences in Asia and Africa [3]. It is the third leading cause of cancer death, and the global burden of HCC continues to increase worldwide [4, 5]. Diethylnitrosamine (DEN) may be a potent hepatocarcinogenic nitrosamine present in tobacco smoke, polluted water, cosmetics, cured meat products, and pharmaceutical agents [6]. DEN-induced HCC is an accepted and widely used experimental model of hepatocarcinogenesis in humans [7].

Plants can be described as nano factories which provide a potential pathway to bio-accumulation into the food chain and the environment. Among the different biological agents, plants provide a safe and beneficial way to the synthesis of metallic nanoparticle as it is easily available so there are possibilities for large-scale production; aside from this, the synthesis route is eco-friendly, and the speed of production is quicker as compared to other biological models such as bacteria, algae, and fungi [8]. Using plant extracts for silver nanoparticle synthesis, a broad variety of metabolites can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The main mechanism considered for the method is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids, flavones, organic acids, of the ions [9]. Green synthesis is safer, eco-friendly, and economic. The role of silver nanoparticles as an anti-cancer agent should open new doors in the field of medicine [10].

Diethylnitrosamine is also bioactivated by cytochrome P450, following which it forms DNA adducts rapidly, bringing about mutation and fragmentation that may lead to the formation of micronuclei. It has been suggested that, on metabolic activation, it produces the pro-mutagenic products, O6-ethyl deoxy-guanosine and O4 and O6-ethyl deoxy-thymidine, in the liver which are responsible for its carcinogenic effect [11]. Cyclophosphamide (CP) is an oxazaphosphorine class of alkylating agent widely used in cancer treatment. Cyclophosphamide (N, N-bis [2chloroethyl]-1, 3, 2-oxazaphosphinan-2-amine 2-oxide; brand name cytoxan) is an anti-cancer alkylating chemotherapeutic drug with immunosuppressive activities. Cyclophosphamide elucidates its toxicity to the cells to DNA alkylating the N7 position of guanine and forming cross link between DNA-DNA and DNA-protein and DNA is single stranded, which ultimately results in loss of normal function of nucleic acids and inhibits DNA synthesis [12]. Since cyclophosphamide also targets the purine bases like guanine in DNA, it will enhance the
treatment of DEN-induced mutation in N7-methylguanine and O6-methylguanine. Hence, cyclophosphamide is selected as a standard drug for this study.

_Cucumis melo_ (L.) is usually referred as musk melon, cantaloupe. They belong to the family of Cucurbitaceae and are cultivated in all tropical regions of the world. They are rich sources of vitamin C, vitamin E, polyphenols, and carotenoids, which have been suggested as natural sources of antioxidants [13]. Many phytochemicals having potential benefits are present in _Cucumis melo_ fruit. The fruits are often used as a cooling, light cleanser or moisturizer for the skin and have stomachic properties. Traditionally, it is used for treatment of kidney stones, cancer, cardiovascular disorders, and stroke [14].

Since the plant, _Cucumis melo_, has been reported to possess antioxidant, anti-inflammatory, immune modulatory, anti-cancer, and hepatoprotective activity [15], there are no systematic scientific studies on the anti-cancer effect of _Cucumis melo_ fruit.

**Materials and Methods**

**Collection and Identification of Plant Material**

_Cucumis melo_ (L.) (Family - Cucurbitaceae) fruits were collected from the local markets of Coimbatore district, Tamilnadu, India. The specimen sample was identified and authenticated by Dr. M. Palanisamy, Scientist-C, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. BSI/SRC/5/23/2014-15/ Tech/482.

**Preparation of Fruit Extract**

The pulp of fresh fruits of _Cucumis melo_ was chopped into pieces and dried at room temperature for 24 h. The air-dried pulps were kept at 40°C in hot air oven for 24 h to remove moisture content. The completely dried fruits were ground into powder by using a mixer grinder and stored. Ten grams of the dried fruit powder was successively extracted with 100 ml of selected solvents (water, methanol, ethanol, chloroform, and ethyl acetate) using soxhlet apparatus and filtered through Whatmann No 1 filter paper. The filtrate was concentrated and dried under reduced pressure and controlled temperature. The concentrated extracts of the fruit were stored in small vials at −20°C and used for further analysis.

**Synthesis of Silver Nanoparticles from Aqueous Extract of Cucumis melo Fruit**

The dried _Cucumis melo_ fruit powder (10 g) was boiled in 100 ml of distilled water for 10 min. The extract was cooled to room temperature, filtered, and used for the synthesis of SNPs. Aqueous solution of 1mM AgNO₃ was prepared and used for the synthesis of silver nanoparticles. Five milliliters of _Cucumis melo_ fruit extract is mixed with 95 ml of AgNO₃ for the synthesis of silver nanoparticles. The synthesized silver nanoparticles are characterized by UV-visible spectroscopy, particle size analyzer, scanning electron microscope, and energy-dispersive spectroscopy, and x-ray diffraction analysis was carried out [16].
Selection of Animals

Healthy adult male Wistar albino rats (age - 3 months) weighing about 150 to 200 g were obtained from Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. They were housed in polypropylene cages under the standard laboratory condition (25 ± 2°C, humidity 60–70%, 12-h light/dark cycles). The animals were fed with commercial rat pellet diet (Sri ram animal foods, Coimbatore) and water was provided ad libitum. The rats were acclimatized to laboratory conditions for 1 week prior to the commencement of the experiment. The animal care and handling were done according to the regulations of Council Directive CPCSEA no: 659/02/a about Good Laboratory Practice (GLP) on animal experimentation. All animal experiments were performed within the laboratory according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC).

Induction of Hepatocellular Carcinoma

The experimental hepatocellular carcinoma was induced by providing 0.01% DEN through drinking water for 16 weeks [17]. It is now widely used as a standard experimental model for HCC [18].

Experimental Design

After 1 week of acclimatization period, the animals were divided into five groups with six animals in each.

**Group I:** Control rats fed with standard diet and water ad libitum.
**Group II:** Rats induced with hepatocellular carcinoma by providing 0.01% DEN through drinking water for 16 weeks.
**Group III:** Rats treated with EECMF (500 mg/kg b.w) orally for 6 weeks after the administration of DEN for 10 weeks.
**Group IV:** Rats treated with SNPs-AECMF intraperitonially (100 μg/kg b.w) for 6 weeks after the administration of DEN for 10 weeks.
**Group V:** Rats treated with standard drug cyclophosphamide (50 mg/kg b.w) orally for 6 weeks after the administration of DEN for 10 weeks.

Collection of Samples

After the experimental regimen (16 weeks), the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected in EDTA and centrifuge tubes by an incision made in the jugular veins and serum was separated by centrifugation at 2000 rpm for 20 min and utilized for various biochemical assays. The liver and kidney were excised immediately and thoroughly washed in ice-cold physiological saline and blotted dry. A part of the tissues such as the liver and kidney were removed and fixed in 10% formalin for histopathological study. Ten percent of liver homogenate
of the washed tissue was prepared in 0.1 M Tris HCl buffer (pH 7.4) and utilized for the biochemical analysis.

Analysis of Biochemical Parameters

Hematological Parameters

The hematological parameters such as hemoglobin, PCV, WBC, RBC, platelets, MCV, MCH, and MCHC were assayed. The whole blood sample was analyzed for the changes in the blood cells using SYSMEX Xs – 800 i automatic hematology analyzer.

Liver Function Markers

The standard methods have been followed to analyze the liver function marker enzymes such as aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, \( \gamma \)-Glutamyl transferase, and 5’-Nucleotidase were analyzed [19–21].

Enzyme-Linked Immunosorbent Assay (ELISA) Of AFP and CEA

The cancer marker enzymes alpha-fetoprotein (AEP) and cancer embryonic antigen (CEA) was analyzed quantitatively using enzyme-linked immune sorbent assay (ELISA) technique [22].

Histopathological Studies—Liver and Kidney

For histopathological examination, the liver and kidney were fixed in 10% formalin and then embedded in paraffin wax. Paraffin-embedded sample blocks were cut into 3–5 m sections using an ultra-microtome and processed for hematoxylin and eosin (H&E) staining. Pathological changes in liver and kidney tissues were evaluated under light microscopy.

Statistical Analysis

Results were expressed as mean ± SD of six animals in each group. Statistical significance \( (p<0.05) \) was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test using SPSS version 17.0.

Results and Discussion

Effect of *Cucumis melo* Fruit and Its Silver Nanoparticles on Hematological Parameters

The blood cells are the mobile units of the body’s protective system. Anemia is frequently occurring in cancer patients [23]. Usually, in cancer chemotherapy, the main problems that are being encountered are of myelosuppression and anemia [24]. Hematological parameters showed a decrease in the levels of Hb, RBC, MCV, MCH, MCHC, and platelets, and concomitant increase in WBC in DEN-induced cancer animals. Significant alterations
in the hematological parameters were observed in EECMF-, SNPs-AECMF-, and cyclophosphamide-treated rats which indicate the protective effect of *Cucumis melo* fruit on the hemopoietic system. Table 1 shows the levels of hemoglobin, lymphocyte count, and RBC count in the control and experimental groups of rats.

The levels of hemoglobin and RBC were found to be significantly \((p < 0.05)\) decreased whereas WBC levels were significantly increased in (Group II) cancer-bearing animals when compared with (Group I) control animals. Co-administration of EECMF and SNPs-AECMF significantly altered the hematological parameters when compared with hepatocellular carcinoma–induced rats. Significant variations were not observed in SNPs-AECMF (Group IV)–treated rats compared to standard drug (cyclophosphamide) rats. Our finding is similar with the study of Althaf and Sudaroli who observed the significant reduction in the total WBC count and increase in the levels of hemoglobin and RBC when treated with ethanolic extract of *Vitex leucoxylon* Linn leaves on hepatocellular carcinoma–induced rats \[25\]. Increased WBC count indicates the decreased resistance of the body to toxicity induced by DEN. Decreased RBC count and hemoglobin also indicate the severity of hepatic damage induced by DEN. Decreased RBC count and hemoglobin can be related to decreased in RBC number which in turn indicates anemic induction \[26\]. Increased RBC, hemoglobin levels, and other parameters indicated the protective effects of EECMF and SNPs-AECMF on the hemopoietic system.

**Effect of *Cucumis melo* Fruit on Liver Function Markers**

The serum biomarkers and biochemical indicators can be an excellent tool or index to evaluate both functional and metabolic aspects of the liver \[27\]. Several enzymes are produced in the liver and are normally distributed within the cells of the liver. Elevation of serum enzyme is taken as the sensitive biomarker of liver toxicity. The determination of various liver enzymes, alanine amino transferase (ALT), aspartate amino transaminase (AST), alkaline phosphatase ALP), \(\gamma\)-glutamyl transpeptidase (\(\gamma\)-GT), and lactate dehydrogenase (LDL) in serum helps to understand the functional status of the liver and to detect liver injury. In the present study, DEN-induced hepatocellular cancer is clearly evidenced by the marked elevation in serum SGPT, SGOT, GGT, and ALP and a decreased level of these enzymes in the liver tissue; these biochemical marker enzymes are indicators of tumor response \[28\]. Serum GGT levels increased linearly with increases in small tumor mass, and ALP levels are elevated in association with small tumors and further increase with increasing tumor mass \[29\].

ALP is used as a specific tumor marker during diagnosis in the early detection of cancer. It is well established that (ALT) levels signify the presence of active disease and increase risk, particularly if the ALT is persistently or intermittently elevated over the years \[30\].

LDH is a cytosolic enzyme that catalyzes the reversible oxidation of L-lactate to pyruvate. Their increased activity in serum confirms increased hepatocyte membrane permeability and cellular leakage. The levels of LDH are strongly correlated with tumor bulk as high rate of glycolysis takes place in cancerous condition, which is the only energy-producing pathway for the malignant cells \[31\].

\(\gamma\)-GT is also believed to be an important indicator and the early enzyme marker of hepatocarcinogenesis. It is located in the outer membrane of the hepatic cells and their elevation reveals cholestasis and bile duct necrosis \[32\].
Table 1  Effect of *Cucumis melo* fruit on hematological parameters of DEN-induced HCC

| Groups                     | HB  ± S.D | PCV ± S.D | WBC ± S.D | RBC ± S.D | Platelets ± S.D | MCV ± S.D | MCH ± S.D | MCHC ± S.D |
|----------------------------|-----------|-----------|-----------|-----------|-----------------|-----------|-----------|-----------|
| Group I (Control)          | 14.00     | 42.00     | 6.51      | 6.00      | 8.48            | 55.50     | 21.83     | 33.16     |
| Group II (DEN induced)     | 9.55 ± 0.36 | 28.33 ± 0.13 | 13.98 ± 0.19 | 3.98 ± 0.14 | 4.46 ± 0.21 | 47.33 ± 1.75 | 18.18 ± 0.42 | 27.08 ± 0.39 |
| Group III (DEN + EECMF)    | 11.20 ± 0.21 | 33.62 ± 0.63 | 9.98 ± 0.54 | 5.50 ± 0.08 | 5.70 ± 0.17 | 50.00 ± 1.41 | 19.51 ± 0.20 | 30.71 ± 1.21 |
| Group IV (DEN + SNPs-AECMF)| 12.33 ± 0.38 | 37.73 ± 1.51 | 7.73 ± 0.12 | 6.05 ± 0.18 | 6.58 ± 0.16 | 52.72 ± 1.23 | 20.45 ± 0.64 | 32.5 ± 0.98 |
| Group V (DEN+Cyclophosphamide) | 13.03 ± 0.16 | 38.85 ± 0.25 | 7.83 ± 0.16 | 6.38 ± 0.14 | 7.08 ± 0.23 | 53.11 ± 0.27 | 21.26 ± 0.16 | 34.05 ± 0.18 |

Values are expressed as mean ± S.D for six animals

Statistical comparison: a - Group II vs Group I, b - Group III vs Group II, Group IV vs Group II, c - Group III vs Group V and Group IV vs Group. The letters a, b, and c represent the statistical significance at p < 0.05.

Units: Hb - g/dl, PCV - %, WBC - thousands/mm³, RBC - millions/mm³, platelets - lakhs/mm³, MCV - fL, MCH - Pg, MCHC - g/dl
5’-nucleotidase (5’NT) is present at the bile canalicular and sinusoidal surface of the plasma membrane of hepatocytes. 5’NT hydrolyzes nucleotides with a phosphate group on carbon atom of the ribose sugar and it was found to be elevated in cancer-bearing animals. In addition, 5’NT is also used as a diagnostic tool for liver injury [33]. In the present investigation, an increased activity of 5’NT activity was observed in hepatocellular carcinoma–bearing animals. The elevation of the marker enzyme may be correlated with the progression of the malignancy and also due to the hepatic cell damage, which may cause leakage of 5’nucleotidase into the circulation.

The activity of AST in the DEN-induced carcinoma rats was found to be significantly increased in serum when compared to the control (Group I) rats. Similar trend was observed in the activities of ALT, ALP, LDH, GGT, and 5’NT in serum of cancer-bearing animals. Co-administration of EECMF- and SNPs-AECMF-treated rats caused a significant decrease in the levels of serum liver marker enzymes when compared to DEN-induced rats (Group II). EECMF-treated rats (Group III) were found to be significantly improved when compared with cyclophosphamide-treated rats whereas significant difference was not observed in silver nanoparticles of aqueous extract of *Cucumis melo* fruit treatment.

Liver cancer–induced rats (Group II) showed significantly (p < 0.05) elevated the levels of these enzymes when compared to control rats. Administration of EECMF- and SNPs-AECMF-treated rats significantly lowered the levels compared to cancer-bearing animals. Significant changes were not observed in the silver nanoparticle–treated rats (Group IV) when compared with standard drug cyclophosphamide–treated rats. The activities of liver marker enzymes, viz. AST, ALT, ALP, LDH, GGT, and 5’NT in the liver of control and experimental rats, are presented in Table 2.

Polyphenolic extract of *Blighia sapidaarilli* fruits at various doses administered to DEN-induced carcinoma rats showed the reduced activities of ALP, ALT, and AST in liver and serum enzymes are reported [34]. Our result agrees well with elevated levels of serum AST, ALT, ALP, LDH, γ-GT, and bilirubin and simultaneous fall in the levels of the marker enzymes in the liver tissue induced by DEN was altered after the administration of ethanolic leaf extract of the medicinal plant *Cassia fistula* Linn [35]. The structural integrity of the cells has been damaged in cancer-induced animals, and this results in cytoplasmic leakage of enzyme into the blood stream, leads to the elevated levels of these enzymes in blood with subsequent fall in the tissues [36].

Hence, EECMF and SNPs-AECMF could have exerted their therapeutic effect against DEN-induced HCC probably by preventing membrane damage and loss of integrity as well as by repairing hepatic tissue damage caused by tumor induction, thus inhibiting the release of these marker enzymes into the serum, indicating that *Cucumis melo* fruit has the ability to prevent further development of HCC.

**Effect of *Cucumis melo* Fruit on the Levels of Cancer Markers**

For hepatocellular carcinoma (HCC), the common method of screening high-risk patients by alpha-fetoprotein (AFP) and ultrasonography has been shown to result in earlier detection [37]. AFP is a 70-kD glycoprotein consisting of 591 amino acids and 4% carbohydrate residues, encoded by a gene on chromosome 4q11-q13. Normally produced during gestation by the fetal liver and yolk sac, AFP is highly elevated in the circulation of newborns with concentrations decreasing during the next 12 months to 10–20 μg/L. Only when serum levels of AFP rise above 500 ng/ml it is sufficiently specific for hepatocellular carcinoma (HCC) [38]. The serum marker, alpha-fetoprotein (AFP), has been used as a diagnostic
Table 2  Effect of *Cucumis melo* fruit on the activities of hepatic marker enzymes in serum of DEN-induced HCC

| Groups                      | AST      | ALT      | ALP      | LDH      | GGT      | 5’NT     |
|-----------------------------|----------|----------|----------|----------|----------|----------|
| Group I (Control)           | 72.14 ± 0.94 | 142.77 ± 1.53 | 17.44 ± 0.62 | 358.78 ± 1.84 | 21.28 ± 1.57 | 3.28 ± 0.21 |
| Group II (DEN induced)      | 41.06 ± 1.53<sup>a</sup> | 129.13 ± 1.56<sup>a</sup> | 32.45 ± 1.32<sup>a</sup> | 432.46 ± 2.06<sup>a</sup> | 36.20 ± 1.27<sup>a</sup> | 7.50 ± 0.14<sup>a</sup> |
| Group III (DEN + EECMF)     | 46.68 ± 2.38<sup>bc</sup> | 136.40 ± 2.07<sup>bc</sup> | 28.02 ± 1.11<sup>bc</sup> | 401.95 ± 5.11<sup>bc</sup> | 31.64 ± 0.98<sup>bc</sup> | 6.58 ± 0.19<sup>bc</sup> |
| Group IV (DEN + SNPs-AECMF) | 56.00 ± 0.87<sup>b</sup> | 147.41 ± 3.42<sup>b</sup> | 26.46 ± 2.08<sup>b</sup> | 363.80 ± 2.00<sup>b</sup> | 28.40 ± 1.68<sup>b</sup> | 5.45 ± 0.18<sup>b</sup> |
| Group V (DEN+Cyclophosphamide) | 57.40 ± 1.06 | 149.30 ± 1.41 | 25.94 ± 1.67 | 365.90 ± 2.97 | 29.87 ± 1.08 | 5.25 ± 0.22 |

Values are expressed as mean ± S.D for six animals

**Statistical comparison:** a - Group II vs Group I. b - Group III vs Group II, Group IV vs Group II. c - Group III vs Group V and Group IV vs Group V. The letters a, b, and c represent the statistical significance at \( p < 0.05 \)

**Units:** AST, ALT, LDH - \( \mu \) moles of pyruvate liberated/min/mg protein, ALP - \( \mu \) moles of phenol liberated/min/mg protein, GGT - \( \mu \) moles of p-nitroanilide liberated/min/mg protein, 5’ NT - \( \mu \) moles of phosphorus liberated/min/mg protein
test for HCC [39]. AFP, as a specific sign of tumor, could provide the important referential value for cancer diagnosis, histopathological classification, and prognosis. AFP content in tumor patient was remarkably higher than the normal tissue near carcinoma and the pre-cancerous lesion tissue [40].

Carcino embryonic antigen (CEA) is an important tumor-associated antigen, and its over expression has been used to identify or diagnose early colorectal, gastric, pancreatic, ovarian cancer and liver cancer. Over expression of CEA in serum always existed in metastatic HCC, and not in primary HCC. CEA are considered cDNA of fatty acid synthesis, tumor necrosis factor specific biomarkers for liver cancer and it is synthesized (TNF) and the house keeping gene-actin as a control, mainly in the fetal stage; practically no production of this were analyzed and the results of gene expression marker occur in the normal adult [41].

The levels of alpha-fetoprotein and carcinoembryonic antigen in the serum of control and experimental rats are given in Fig. 1.

Hepatoma-bearing rats (Group II) possessed increased levels of alpha-fetoprotein (7.5 ng/ml) and CEA (4.6 ng/ml) when compared to control rats. Treatment with EECMF (Group III) and SNPs-AECMF (Group IV) decreased the levels of AFP (4.3 ng/ml) and CEA (3.1 ng/ml) when compared to DEN-induced HCC rats (Group II). Treatment with the EECMF and SNPs-AECMF caused a significant depletion in the levels of AFP and significant difference was not recorded when compared to standard drug-treated rats. Administration of limonin isolated from orange and lemon seeds decrease the tumor marker AFP levels might against N-nitrosodiethylamine (DEN, 200 mg/kg) induced phenobarbital promoted experimental hepatocellular carcinoma in male Wistar albino rats. Morin (3, 5, 7, 2’, 4’-pentahydroxyflavone), a plant-derived flavonoid, significantly lowered the AFP and CEA levels in DEN-induced cancer rats [42].

Exposure of rats to certain carcinogens like DEN causes an elevation of circulatingAFP level. CEA is cleared from the circulation by the liver with significant traces taken up by the spleen and lungs. An increase in serum CEA levels upon DEN treatment was
presumably associated with production rates of tumor, its location and stage, size, differentiation, and vascularity [43]. Upon liver injury, these enzymes enter into the circulatory system due to altered permeability of the membrane. The observed reduction in the levels of AFP and CEA in EECMF- and SNPs-AECMF-treated animals might be due to a decrease in the rate of tumor development, indicating their anti-cancer activity because of the presence of alkaloids, flavonoids, terpenoids, and other phytochemicals of Cucumis melo fruit extract [44].

**Histopathological Investigations of the Liver and Kidney**

Microscopic observations of Cucumis melo fruit–treated DEN-induced rat liver are given in Fig. 2 that shows the histopathological examination of the liver and kidney. Control (Group I) rats revealed normal liver hepatocytes with granulated cytoplasm, small uniform nuclei, and central vein surrounded by cords of hepatocytes and normal architecture. Group II (DEN-induced) rats showed loss of architecture and lobules of neoplastic hepatocytes with a focal area of fatty change. Group III (EECMF) rats exhibited DEN showed a moderate cancerous change, fatty change, and hydropic degeneration. Groups IV (SNPs-AECMF) and Group V (standard drug) rats showed fewer neoplastically transformed cells and the hepatocytes maintained near-normal architecture.

The histological study of rat kidneys showing Group I renal glomerular tubules and normal architecture. Group II (DEN-induced HCC rats) showing blood vessel congestion and inflammatory infiltration. Group III (EECMF-treated rats) shows moderate renal glomeruli congestion of interstitial blood vessels and hemorrhage with minimal inflammatory infiltration. Group IV (SNPs-AECMF) and Group V (Cyclophosphamide) treated rats show mild glomeruli congestion with narrow focal dilation and mild inflammatory infiltration.

**Fig. 2** Histopathological architecture of the liver. Histopathological architecture of the kidney (H&E, ×200, scale bar = 20 µm)
Histopathological investigation of the liver showed loss of architecture and lobules of neoplastic hepatocytes with a focal area of fatty change in DEN-induced liver cancer rats. These changes were found to be reduced tremendously in EECMF-, SNPs-AECMF-, and standard drug cyclophosphamide–treated animals. The kidney of cancer-induced rats showed tubular damage and portal inflammation within due to glomerular damage. The kidney of treated rats with EECMF and SNPs-AECMF showed the mild portal inflammation. Treatment with *Cucumis melo* fruit extract showed the improvement in the architecture of the liver and kidney of cancer rats which might be due to the reduction in the cancerous condition of the organs.

**Conclusion**

The ethanolic extract and silver nanoparticles of aqueous extract of *Cucumis melo* fruit possess potent antioxidant and anti-cancer activities. The anti-cancer activity of the silver nanoparticles of aqueous extract of *Cucumis melo* fruit was similar to that of rats treated with standard drug cyclophosphamide, proving the effective anti-cancer potential against DEN-induced hepatocellular carcinoma in rats. Silver nanoparticles of *Cucumis melo* fruit extract may easily enter into the cancer cells which are having larger pore size compared to normal cells. Hence, size-controlled targeting of silver nanoparticles can prove their effectiveness in cancer treatment, might be due to the morphological differences between cancer and the other normal cells. Silver nanoparticles of aqueous extract of *Cucumis melo* fruit were found to exhibit the anti-cancer effect only on the tumor cells (chemotherapeutic effect) but not on the normal cells revealed by toxicity studies (pharmacodynamic effect). The present investigation conclusively substantiates that the anti-cancer potential of ethanolic extract and silver nanoparticles of *Cucumis melo* fruit might be due to the additive and synergistic effects of the secondary metabolites such as alkaloids, flavonoids, terpenoids, and other phytochemicals of *Cucumis melo* fruit. The molecular mechanism of silver nanoparticles inhibits the tumor growth has yet to be explored.

**Abbreviations**

EECMF: Ethanol extract of *Cucumis melo* fruit; SNPs-AECMF: Silver nanoparticles of aqueous extract of *Cucumis melo* fruit; ACP: Acid phosphatase; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; EDTA: Ethylene diamine tetra acetic acid

**Declarations**

**Conflict of Interest** The authors declare that they have no conflict of interest.

**References**

1. Thevasundari, S., & Rajendran, A. (2011). Antibacterial potential and phytochemical analysis of *Heterostemma tanjorense* (Wight and Arn). *World Journal of Science and Technology, 1*(11), 39–45.
2. Kawasaki, E. S., & Player, A. (2005). Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer. *Nanomedicine, 1*, 101–109.
3. Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians, 61*, 69–90.
4. Wei, Q., Mu, K., Li, T., Zhang, Y., Yang, Z., Jia, X., Zhao, W., Huai, W., Guo, P., & Han, L. (2014). Deregulation of the NLRP3 inflammasome in hepatic parenchymal cells during liver cancer progression. Laboratory Investigation, 94(1), 52–62.

5. Zhu, X. L., Wang, Y. L., Chen, J. P., Duan, L. L., Cong, P. F., Qu, Y. C., Ling, J. L., & Zhang, M. X. (2014). Alternor inhibits migration and invasion of human hepatocellular carcinoma cells by targeting epithelial-to-mesenchymal transition. Tumour Biology, 35(2), 1627–1635.

6. Sun, H., Yu, L., Wei, H., & Liu, G. (2012) A novel antihepatitis drug, bicyclol, prevents liver carcinogenesis in diethylnitrosamine initiated and phenobarbital-promoted mice tumor model. Journal of Biomedicine and Biotechnology 584–728.

7. Sivaramakrishnan, V., Shilpa, P. N., Praveen-Kumar, V. R., & Niranjali-Devaraj, S. (2008). Attenuation of N-nitrosodiethyamine induced hepatocellular carcinogenesis by a novel flavonol- Morin. Chemico-Biological Interactions, 171, 79–88.

8. Nour, A. E. M., Eftaiha, A., Al-Warthan, A., & Ammar, R. A. (2010). Synthesis and applications of silver nanoparticles. Arabian Journal of Chemistry, 3, 135–140.

9. Jha, A. K., Prasad, K., Prasad, K., & Kulkarni, A. R. (2009). Plant system: Nature’s nanofactory. Colloids and Surfaces B: Biointerfaces, 73, 219–223.

10. Devi, J. S., & Bhimba, B. V. (2012). Anticancer activity of silver nanoparticles synthesized by the seaweed Ulva lactuca in vitro. Open Access Science Report, 1(4), 1–4.

11. Prasad, S. B., Nicol, B. M., & RosangkimaAmenla, G. (2010). Modulatory effect of ascorbic acid (vitamin C) on cyclophosphamide-mediated antitumor activity and mutagenicity in mice bearing ascites daltons lymphoma. International Journal of Pharma and Bio Sciences, 1(2), 1–20.

12. Magee, P. N., & Farber, E. (1962). Toxic liver injury and carcinogenesis. Methylation of rat-liver nucleic acids by dimethylnitrosamine in vivo. The Biochemical Journal, 83, 114–124.

13. Lester, G. E., Jifon, J. L., & Crosby, K. M. (2009). Superoxide dismutase activity in mesocarp tissue from divergent Cucumis melo L. genotypes. Plant Foods for Human Nutrition, 64(3), 205–211.

14. Ritschel, P. S., Lins, T. C., Tristan, R. L., Buso, G. S., Buso, J. S., & Ferreira, M. E. (2004). Development of microsatell markers from an enriched genomic library for genetic analysis of melon (Cucumis melo L.). BMC Plant Biology, 4, 9.

15. Parlie, M., & Singh, K. (2011). Musk meol is eat must-melon. International Journal of Pharmaceutical Science and Research, 2(8), 52–57.

16. Vidya, R., & Kalaivani, K. (2020). Green synthesis of silver nanoparticles and its characterization using Cucumis melo (L). Fruit extract. International Journal of Botany Studies, 5(5), 99–103.

17. Ramakrishnan, G., Raghavendran, H. R., Vinodhkumar, R., & Devaki, T. (2006). Suppression of N-nitrosodiethyamine induced hepatocarcinogenesis by silymarin in rats. Chemico-Biological Interactions, 161, 04–114.

18. Sivaramakrishnan, V., & Devaraj, S. N. (2009). Morin regulates the expression of NF-κ B-p65, COX-2 and Matrix metalloproteinases in diethylnitrosamine induced rat hepatocellular carcinoma. Chemico-Biological Interactions, 180, 353–359.

19. King, E. J., & Armstrong, A. R. (1934). Estimation of alkaline phosphatase. Canadian Medical Association Journal, 311, 152–156.

20. Reitman, S., & Frankel, S. A. (1957). Calorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. American Journal of Clinical Pathology, 28(1), 56–63.

21. King (1954). In the hydrolases: Acid and alkaline phosphatase. Practical Clinical Enzymology. London: Kerstin Company Ltd. pp. 191–208.

22. Sell, S., & Becker, F. F. (1978). Alpha fetoprotein. National Cancer Institute, 60(1), 19–26.

23. De Vita, V. T., Hellman, S, & Rosenberg, S. A. (1993). (Editors). Cancer: Principle and practice of oncology. 4th Edition. JB. Lippincott Company, Philadelphia.

24. Hogland, H. C. (1982). Hematological complications of cancer chemotherapy. Seminars in Oncology, 9, 95–102.

25. AlthafFaimumSudaroli, D. (2012). Influence of Vitex Leucoxylon Linn on oxidative stress and hepatocarcinogenesis induced by diethylnitrosamine and phenobarbital in rats. International Journal of Toxicology and Pharmacology Research, 4(4), 96–107.

26. Sarantchandra, G., Chandre, J., Jayasuder, S., & Murthy, P. B. (1996). Toxicology of Cleistanthus collinus an indigenous plant acute toxicity study. Indian Journal of Toxicology, 3, 9–17.

27. Ingawale, D. K., Mandlik, S. K., & Naik, S. R. (2014). Models of hepatotoxicity and the underlying cellular, biochemical and immunological mechanism(s): A critical discussion. Environmental Toxicology and Pharmacology, 37, 118–133.

28. Carr, B. I., Lu, S. N., & Pancoska, P. (2011). Small hepatocellular carcinoma in Chinese patients. Hepato-gastroenterol, 58(109), 1334–1342.
29. Pancoska, P., De Giorgio, M., Fagiuoli, S., & Carr, B. I. (2011). Small HCCs identified by screening. *Digestive Diseases and Sciences, 56*(10), 3078–3085.
30. Sherman, M. (2009). Risk of hepatocellular carcinoma in hepatitis B and prevention through treatment. *Cleveland Clinic Journal of Medicine, 76*(3), 6–9.
31. Nandakumar, N., Jayaprakash, R., Rengarajan, T., Ramesh, V., & Balasubramanian, M. P. (2011). Hesperidin, a natural citrus flavonoglycoside, normalizes lipid peroxidation and membrane bound marker enzymes in 7, 12-Dimethylbenz (a) anthracene induced experimental breast cancer rats. *Biomedicine & Preventive Nutrition, 1*, 255–262.
32. Reynaert, H., Vaeyens, F., Qin, H., Hellemans, K., Chatterjee, N., Winand, D., Quartier, E., Schuit, F., Urbain, D., Kumar, U., Patel, Y. C., & Geerts, A. (2001). Somatostatin suppresses endothelin-1-induced rat hepatic stellate cell contraction via somatostatin receptor sub type 1. *Gastroenterology, 121*, 915–930.
33. Fredericks, W. M., Cornelis, J. F., Noorden, V., Aronson, D. C., Maex, F., Bosch, K. S., Jonges, G. N., Vogels, I. M., & James, J. (1990). Quantitative changes in acid phosphatase, alkaline phosphatase and 5'-nucleotidase activity in rat liver after experimentally induced cholestasis. *Lever, 10*, 158–166.
34. Oloyede, O. B., Taofeek, O. A., & Yesirat, O. K. (2013). N-nitrosodiethylamine induced redox imbalance in rat liver: Protective role of polyphenolic extract of *Blighia sapida arilli*. *Free Radicals and Antioxidants, 3*, 25–29.
35. Pradeep, K., Rajmohan, C. V., Gobianand, K., & Karthikeyan, S. (2010). Protective effect of *Cassia fistula* Linn. on diethylnitrosamine induced hepatocellular damage and oxidative stress in ethanol pretreated rats. *Biological Research, 43*, 113–125.
36. Thapa, B. R., & Walia, A. (2007). Liver function tests and their interpretation. *Indian Journal of Pediatrics, 74*(7), 663–671.
37. Pournima, M., Seema, M. J., Manisha, K., & Vilasrao, K. (2012). Biomarkers in Oncology *Journal of Applied Pharmaceutical Sciences, 2*(3), 182–191.
38. Tan, H. T., Low, J., Lim, S. G., & Chung, M. C. M. (2009). Serum autoantibodies as biomarkers for early cancer detection. *FEBS Journal, 276*(23), 6880–6904.
39. Debruyne, E. N., & Delanghe, J. R. (2008). Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: New aspects and applications. *Clinica Chimica Acta, 395*, 19–26.
40. Marchesi, M. C., Conti, M. B., Pieramati, C., Mangili, V., & Fruganti, G. (2007). Assessment and behavior of Alphafetoprotein (AFP), Antigen Cancer15/3 (CA15/3), Carcinembryonal Antigen (CEA) in clinical oncology of the dog: Preliminary study. *Veterinary Research Communications, 31*, 301–304.
41. Ragab, G. M. A., El-Denshary, E. S., Hassan, A. M., Abdel- Azaim, S. H., Hassan, N. S., Manna, F. A., & Abdel-Wahhab, M. A. (2013). Grape (*Vitis vinifera*) seed extract inhibits the cytotoxicity and oxidative stress in liver of rats treated with carbon tetrachloride. *Global Journal of Pharmacology, 7*(3), 258–269.
42. Subbaraj, K. L., Srinivasan, G. K., Rajendran, P. R., & Maruthaiveeran, P. (2013). Limonin-A citrus limonoid, establish anticancer potential by stabilizing lipid peroxidation and antioxidant status against N-nitrosodiethylamine induced experimental hepatocellular carcinoma. *Biomedical Preventive Nutrition, 3*, 165–171.
43. Zimmer, R., & Thomas, P. (2001). Mutations in the carcinoembryonic antigen gene in colorectal cancer patients: Implications on liver metastasis. *Cancer Research, 61*, 2822–2826.
44. Al-Atar, A. M. (2011). Hepatoprotective influence of vitamin C on thioacetamide-induced liver cirrhosis in Wistar male rats. *Journal of Pharmacology and Toxicology, 6*, 218–233.

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