

EFFECT OF *SOLANUM VILLOSUM* (MILL.) EXTRACT AND ITS SILVER NANOPARTICLES ON HEMATOPOIETIC SYSTEM OF DIETHYLNITROSAMINE-INDUCED HEPATOCELLULAR CARCINOMA IN RATS

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ABSTRACT

Objective: This study aims to elucidate the different forms of *Solanum villosum* (SV), ethanolic leaf extract of SV (EESV), and SV silver nanoparticles (SV-AgNPs) on hematological parameters in diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC) in rats.

Methods: A total of 30 male albino Wistar rats were randomly assigned into five groups of six rats each. Group 1 was control, whereas Groups 2 (DEN control), Group 3 (EESV 200 mg/kg b.w), Group 4 (SV-AgNPs 100 µg/kg b.w i.p), and Group 5 (cyclophosphamide 50 mg/kg. b.w), orally for 16 weeks, respectively. Rats in all six groups received normal pallet diet and drinking water ad libitum. Complete blood count was done using SYSMEX Xs-800i automatic hematology analyzer.

Results and Discussion: Rats treated with DEN showed severe toxic symptoms and significant decrease in ($p < 0.05$) 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) counts. After, the administration of EESV and SV-AgNPs showed a protective effect on hematopoietic levels of DEN-induced rats.

Conclusion: Thus, it is concluded that oral administration of ethanolic EESV and SV-AgNPs caused an increased in the level of Hb concentration, PCV, RBC, MCV, MCH, MCHC, and PLTS, whereas the level of WBCs was reduced in DEN-induced HCC.

Keywords: Ethanolic extract of *Solanum villosum*, *Solanum villosum* silver nanoparticles, Diethylnitrosamine, White blood cells.

INTRODUCTION

Hepatocellular carcinoma (HCC) is 1 of the 10 most common human cancers, with a worldwide incidence of over one million cases every year. It accounts for about 90% of all primary liver cancers. HCC, a fatal malignancy represents 4% of all malignant tumors. Most primary liver cancers are classified as HCC [1,2]. Primary liver cancer is one of the most common cancers in the world, accounting for an estimated 600,000 deaths annually [3]. In Korea, liver cancer is the second leading cause of cancer-related deaths (10,946 death in 2010) [4]. In the United States, primary liver cancer has gained major interest because the incidence of liver cancer has increased over the past 25 years, and the incidence and mortality rate of liver cancer are expected to double over the next 10-20 years [5-7].

More than 60% of the world's total cases occur in Africa, Asia, and Central and South America, and these regions account for about 70% of the world's cancer deaths, a situation that is made worse by the lack of early detection and access to treatment [8]. In the United States and Europe, secondary (metastatic) liver tumors are more common than primary liver cancer. The opposite is true for many areas of Asia and Africa [9].

Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single electron transistors, light emitters, nonlinear optical devices, and photoelectrochemical applications [10-12].

Traditionally, plants have been well exploited by man for the treatment of human diseases. The Indian subcontinent is a rich source of plant

and animal wealth, which is due to its varied geographical and agroclimatic regions [13]. *Solanum villosum* (SV) (Mill) belongs to family Solanaceae; it is commonly known as red-fruit nightshade and is widely distributed in many parts of India. The plant is an ayurvedic herb with multiple medicinal properties [14,15]. No studies have been reported on this plant to assess the activity against the HCC in rats.

METHODS

Collection and processing of plant material

The plant, SV (Mill), was collected from Thadagam hills at Coimbatore district, Tamil Nadu, India. The specimen sample was identified and authenticated by Dr. Murthy, Joint Director, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. The identification No. BSI/SRC/5/23/2014-15/Tech/255. The leaves were shade dried and powdered using mixer grinder. The powdered material (10 g) was extracted with 100 ml of ethanol using Soxhlet apparatus and filtered. The filtrate was concentrated and dried under reduced pressure and controlled temperature.

Synthesis of silver nanoparticles (AgNPs)

The dried SV leaves powder 10 g was boiled in 100 ml of distilled water for 10 minutes. The extract was cooled to room temperature filtered and used for the synthesis of AgNPs. Aqueous solution of 1 mM AgNO₃ was prepared and used for the synthesis of AgNPs. 5 ml of aqueous SV aqueous extract is mixed with 95 ml of AgNO₃ for the synthesis of AgNPs. The formation of AgNPs is confirmed by color change from greenish to reddish brown. The appearance of reddish brown color after 3 hrs indicates the formation of AgNPs. The synthesized AgNPs are characterized by UV-visible spectroscopy, scanning electron microscope, and X-ray diffraction analysis were carried out.

Experimental animals

Healthy adult male Wistar albino rats weighing about 150-200 g were obtained from the small animal breeding center, 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 hrs light/dark cycles). The animals were fed with commercial rat pellet diet (Sriram feeds, Coimbatore), and water was provided *ad libitum*. The rats were acclimatized to laboratory conditions for 1 week before 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 on animal experimentation. All animal experiments were performed in the laboratory according to the ethical guidelines suggested by the Institutional Animal Ethics Committee.

Chemicals

N-nitrosodiethylamine was purchased from Sigma Laboratories, USA. All chemicals used in the study were of analytical grade and obtained from precision diagnostics.

Experimental design

Group I: Control rats fed with standard diet and water *ad libitum*.

Group II: Rats induced with HCC by providing 0.01% diethylnitrosamine (DEN) through drinking water for 16 weeks.

Group III: Rats treated with rats treated with EESV (200 mg/kg b.wt) orally for 6 weeks after the administration of DEN for 10 weeks.

Group IV: Rats treated with SNPs-AESVL intraperitoneally (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.

After administering DEN alone for 10 weeks, the rats were treated with SNPs-AESVL and cyclophosphamide along with DEN for another 6 weeks respectively.

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. For hematological study hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), and platelet, blood was collected in ethylenediaminetetraacetic acid-coated tube by an incision made in the jugular veins.

Hematological assay

The hematological parameters such as Hb, PCV, RBC, WBC, MCV, MCH, MCHC, and platelet counts were assayed. The whole blood sample was analyzed for the changes in the blood cells using SYSMEX Xs-800i (5 part) automatic hematology analyzer.

Statistical analysis

Results were expressed as mean±standard deviation of six animals in each group. Statistical significance was determined by one-way analysis of variance and *post-hoc* least-significant difference test using SPSS 17 version.

RESULTS AND DISCUSSION

WBC count of DEN-induced rats ($13.98 \pm 0.19 \times 10^3/\mu\text{l}$) showed a significant increase ($p < 0.05$) as compared to normal control rats ($6.51 \pm 0.11 \times 10^3/\mu\text{l}$) is may due to uncontrolled proliferation of cancerous cells or immunity response against the invading cancer cells. The treatment Groups III, IV, and V showed a significant decrease of WBC count of cancer rats compared to DEN-induced groups; the recorded values were 9.65 ± 0.18 , 7.51 ± 0.23 , and $7.13 \pm 0.16 \times 10^3/\mu\text{l}$ for EESV, SV-AgNPs, and cyclophosphamide, respectively (Table 1).

Increased total leukocyte count in the animals of indicates decreased resistance of the body to toxicity induced by DEN [16]. Decreased RBC count, Hb also indicates the severity of hepatic damage induced by DEN. Decrease in the Hb levels might be due to increased catabolism and

degradation of Hb. Reduction in Hb content can be related to decrease in RBC number which, in turn, indicates anemic induction [17,18]. A change in WBC counts commonly results from an infection, a malignancy, anticancer drug use, a drug allergy, or a hematological disease [19].

In the present study, significantly decreased levels of Hb, PCV, RBC, MCV, MCH, MCHC, and platelets (PLTS) in Group II (DEN) were noted when compared to normal controls (Figs. 1-3). The same results are

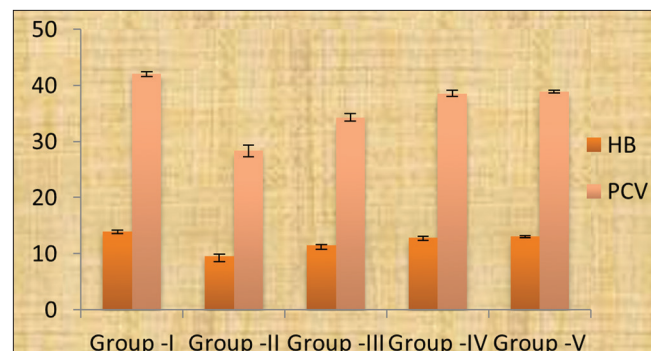


Fig. 1: Effect of ethanolic leaf extract of *Solanum villosum* and silver nanoparticles of *S. villosum* (Mill.) on hemoglobin and packed cell volume of diethylnitrosamine-induced hepatocellular carcinoma in rats

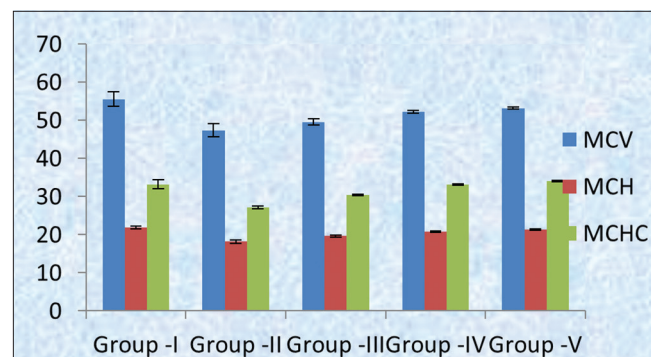


Fig. 2: Effect of ethanolic leaf extract of *Solanum villosum* and silver nanoparticles of *S. villosum* (Mill.) on red blood cells, white blood cell and platelets of diethylnitrosamine-induced hepatocellular carcinoma in rats

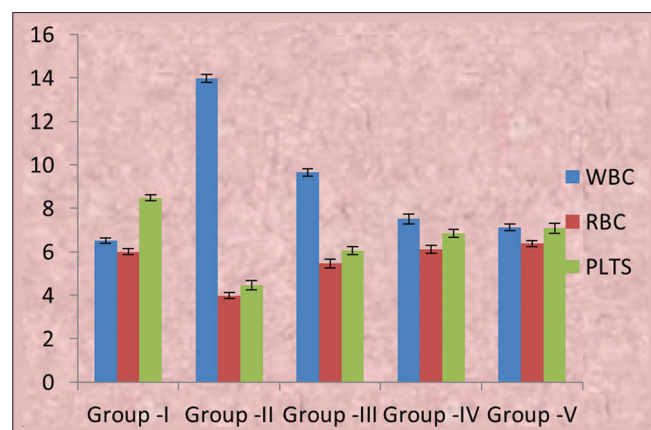


Fig. 3: Effect of ethanolic leaf extract of *Solanum villosum* and silver nanoparticles of *S. villosum* (Mill.) on mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration of diethylnitrosamine-induced hepatocellular carcinoma in rats

Table 1: Effect of EESV and AgNPs of SV (Mill.) on hematological parameters against DEN-induced HCC in rats

Hematology	HB	PCV	WBC	RBC	PLTS	MCV	MCH	MCHC
Group I	14.00±0.14	42.00±0.42	6.51±0.11	6.00±0.14	8.48±0.14	55.50±1.87	21.83±0.43	33.16±1.16
Group II	9.55±0.36*	28.33±1.03*	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	11.45±0.20*	34.25±0.68*	9.65±0.18*	5.46±0.21*	6.06±0.19*	49.48±0.83*	19.60±0.26*	30.45±0.18*
Group IV	12.86±0.20*	38.55±0.56*	7.51±0.23*	6.11±0.19*	6.85±0.18*	52.18±0.43*	20.75±0.18*	33.16±0.21*
Group V	13.03±0.16	38.85±0.25	7.13±0.16	6.38±0.14	7.08±0.23	53.11±0.27	21.26±0.16	34.05±0.18

Hb: g/dl, PCV: %, WBC: Thousands/mm³, RBC: Millions/mm³, PLTS-lakhs/mm³, MCV: fL, MCH: Pg, MCHC: g/dl. Values are expressed as mean±SD for six animals.

Statistical comparison: Group I and Group II; Group II and Group III; Group II and Group IV. The *represents the statistically significant at P<0.05. The letter ns represent the nonsignificance. SD: Standard deviation, EESV: Ethanolic leaf extract of *Solanum villosum*, AgNPs: Silver nanoparticles, SV: *Solanum villosum*, HB: Hemoglobin, PCV: Packed cell volume, WBC: White blood cells, RBC: Red blood cells, PLTS: Platelets, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

obtained in the thyroid cancer patients reported by Bircan *et al.* [20]. After administration of ethanolic leaf extract of SV (EESV), aqueous leaf extract of SV-AgNPs, and standard drug cyclophosphamide, the above hematological parameters increased significantly in Group III, IV, and V experimental groups. Compare to ethanolic leaf extract, the AgNPs have the more effect is may be due to protective effect of the combination of plant and AgNPs on the hematopoietic system.

The smaller the diameter of the nanoparticles is the more its influence to cells and its molecular effects on the intracellular mechanisms will increase. Due to the higher contact surface and more influence on the cell membrane in higher doses, nanosilver particles lead to the influence in WBC mitochondria and changes in their enzyme activity.

The WBC count is a measurement of the cells of the blood that the body uses to fight infection and react against foreign bodies or tissues. The percentages of the five different types of WBCs may temporarily shift depending on body functions. A high WBC count may indicate acute infection, inflammation, or tissue damage. RBCs are concave shaped cells that are filled with Hb, the protein that transports oxygen and carbon dioxide throughout the body. The hematocrit is a measurement of the proportion of the blood that is made of RBCs. The Hb concentration is the measure of the total amount of Hb in the blood. MCV, MCH, and MCHC are red blood cell parameters. They tell about red blood cell size, Hb concentration per cell, and size variation. These parameters are only important when interpreted along with Hb, hematocrit, and red blood cell count. PLTS are the tiny cells that play an essential role in blood clotting. They are the first components to be activated when there has been an injury to a blood vessel and begin the formation of a blood clot. There is a risk of excessive bruising and bleeding when there are not enough PLTS.

Assessment of hematological parameters can be used to determine the effect of the foreign compound including plant extract and AgNPs on the blood. It can be used to explain blood relating functions of plant extract [21]. The slight insignificant (p>0.05) decrease in the red blood cell and Hb may have resulted from the suppression of circulating hormone, erythropoietin (a glycoprotein which stimulates the process of erythropoiesis) [22]. Reduction in blood concentration of erythropoietin may result in a normochromic, normocytic anemia [23].

Actually, assessments of hematological parameters are used to determine the extent of deleterious effect of the extracts on blood of an animal [24,25] reported that reduction in RBC, Hb, and PCV is an indication of either the destruction of RBC or their decreased production, which may lead to anemia. On the contrary an increase in the count of RBC, Hb, and PCV is suggestive of polycythemia and positive erythropoiesis [26-28]. Reports about WBC counts have pointed out that whereas increased count of WBC is supposed to be helpful in boosting immune system [29,30], a decreased count of WBC shows the suppression of leukocytes and their production from bone marrow [31-33]. Anemia, however, is very common so that only a small minority of patients with anemia have colon cancer. Iron deficiency anemia of undetermined etiology, however, warrants evaluation for colon cancer, particularly in the elderly [34].

Biological methods for nanoparticle synthesis using plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods [35]. Nanotechnology is an attractive area of research related to the production of nanoparticles of variable size, shape, and chemical composition, with controlled dispersity, as well as their possible benefits in clinical medicine [36]. Biosynthesis of nanoparticles, as a representative intersection of nanotechnology and biotechnology, has been the focus of increasing attention due to the growing need to develop environmentally friendly technologies for materials synthesis [37].

Moreover, when AgNPs are injected intravenously, they interact initially with the blood and its components, and they may cause various immunogenic responses, inflammation, and changes in hematological parameters, including white cells and PLTS [38]. The changes in white and RBC reported here after the first injection of nanoparticles have been described before and are possibly due to an increased immunogenic response [39,40] or disturbances in signaling pathways and maturation of cells, [41] which can affect RBC as well as the division and development of other cells.

CONCLUSION

In conclusion, the result of this study suggests that EESV + SV-AgNPs have a positive, beneficial effect against the chemically induced hepatocarcinogenesis in rats, which provides an effective chemopreventive combination approach to manage the disease. Moreover, the results suggest that both EESV and SV-AgNPs are safe and capable of normalizing hematological abnormalities cancer associated anemic condition. However, further studies are needed with regard to other bioassays and documentation of specific molecular markers to establish the exact mechanism for EESV+SV-AgNPs mediated chemoprevention of cancer.

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