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# Attenuation of DEN induced hepatocellular carcinoma by a novel synthesis of silver nanoparticles using *Solanum villosum* (Mill.) leaves

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## ABSTRACT

**Objective:** In today's fast and furious life, the life style and food habits of people are changing drastically, due to which ninety percent of the population suffers with oxidative stress resulting as ageing process, arthritis, ischemia, neurodegeneration, chronic liver diseases and cancer. The aim of the study is to evaluate the anticancer potential of *Solanum villosum* leaves against the diethylnitrosamine induced experimental hepatocellular carcinoma in male wistar albino rats.

**Methods:**Various biochemical, cancer markers, intermediatery enzymes and histopathology were studied systematically for different groups of rats.

**Results:** The animals exposed to diethylnitrosamine showed significant alterations in all biochemical and metabolic enzymes. After administration of silver nanoparticles of *Solanum villosum* leaves (SNPs-AESVL 100 µg/kg b.w) effectively suppressed the tumor growth induced by diethylnitrosamine.

**Conclusion:** Our findings suggest that silver nanoparticles of *Solanum villosum* leaves are potential anticancer effect, preventing tumor growth and hepatocellular damage.

**Keywords:** *Solanum villosum*, Diethylnitrosamine (DEN), Hepatocellular carcinoma, silver nanoparticles, Cyclophosphamide.

## INTRODUCTION

Cancer nanotechnology is an upcoming branch of nanotechnology that has emerged recently as one of the most propitious fields in cancer treatment. Biosynthesized silver nanoparticles have a wide range of biological activities such as antitumor, antifungal, apoptosis, interaction with DNA, thereby inhibiting replication, transcription, and other nuclear functions and arresting cancer cell proliferation to arrest tumor growth. Using nanoparticles for early detection, accurate diagnosis, and tailored treatment of cancer as one of the novel approaches in cancer therapy.

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer death, and accounts for 5.6 % of all cancers [1-2]. Nearly 82 % of the approximately 550,000 liver cancer deaths each year occur in Asia [3]. Despite significant advances in medicine, liver cancer, predominantly hepatocellular carcinoma remains a major cause of death in the United states as well as the rest of the world [4-5]. As limited treatment options are currently available to patients with liver cancer, novel preventive control and effective therapeutic approaches [6] are considered to be reasonable and decisive measures to combat this disease.

*Solanum* is one of the most important and largest genera of the family Solanaceae comprising of about 84 genera and 3000 species were identified throughout the worldwide. The plant is an ayurvedic herb with multiple medicinal properties

[7]. The ethnobotanical survey also reported that the wild edible plants traditionally used as leafy vegetables by the tribes in Tamilnadu, Kerala, India [8-9]. Based on our literature survey, no studies have been carried out on this plant to assess its anticancer activity. So evaluation of anticancer potential of *Solanum villosum* (Mill.) silver nanoparticles against DEN induced hepatocellular carcinoma in rats is a novel approach in the field of cancer biology.

## MATERIALS AND METHODS

**Plant material collection and identification**Fresh plant leaves, *Solanum villosum* (Mill.) was collected from Thadagam hills at Coimbatore district, Tamil Nadu, India. The specimen sample was identified and authenticated from Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. is BSI/SRC/5/23/2014-15/Tech/255.

**Synthesis of silver nanoparticles from silver nanoparticles of aqueous extract of *Solanum villosum* leaves (SNPs-AESVL)**

The dried *Solanum villosum* 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 SNPs. Aqueous solution of 1mM $AgNO_3$ was prepared and used for the synthesis of silver nanoparticles. 5 ml of *Solanum villosum* aqueous extract is mixed with 95 ml of $AgNO_3$ for the synthesis of silver nanoparticles. The synthesized silver nanoparticles are characterized by UV-visible spectroscopy, particle size analyzer, scanning electron microscope, Energy dispensive spectroscopy, x-ray diffraction analysis were carried out [10-11].

**Experimental animals**

Healthy adult male wistar albino rats weighing about 150 to 200 g were obtained from small animal breeding center, Kerala agricultural university, Mannuthy, Thrissur, India. They were housed in polypropylene cages (38×23×10 cm) under the standard laboratory condition (25 ± 2°C, humidity 60-70 %, 12 hours light / dark cycles). The animals were fed with commercial rat pellet diet (Sriram animal foods, Coimbatore, Tamil Nadu, India.) and water was provided *ad libitum*. The rats were acclimatized to laboratory conditions for one week prior to the commencement of the experiment. All animal experiments were performed in the laboratory according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (Approval No: 659/02/a/CPCSEA).

## Chemicals

Diethynitrosamine (DEN) was purchased from Sigma Laboratories, (St. Louis, MO, USA) and biochemical reagents was purchased by precision chemicals, Coimbatore, India. All chemicals used in the study were of analytical grade.

## Induction of hepatocellular carcinoma

The experimental hepatocarcinogenesis was induced by providing 0.01% DEN (Sigma , USA) through drinking water for 16 weeks as described by [12-13].

## Selection of therapeutic doses

Based on toxicity studies, different doses of silver nanoparticles of aqueous extract of *Solanum villosum* leaves (SNPs-AESVL) ranges from 100 µg, 200 µg and 300 µg/kg body weight were treated for 28 days in rats. Finally the effective dose (100 µg/kg) was fixed based on the Hematological, biochemical and histopathological studies are previously published [14].

## Experimental design

| | |
|---|---|
| **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 SNPs-AESVL intraperitonially (100 µg / kg b.w) for 6 weeks after the administration of DEN for 10 weeks. |
| **Group IV** | 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 six weeks respectively.

## 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 minutes and utilized for various biochemical assays. The liver and spleen were excised immediately and thoroughly washed with ice cold physiological saline and blotted dry. A part of the tissues such as liver and spleen were removed and fixed in 10 % formalin for histopathological study. 10 % of liver homogenate of the washed tissues were prepared in 0.1 M Tris HCl buffer (pH 7.4) and utilized for the biochemical analysis.

## Biochemical assays

Serum and liver homogenate was prepared and used for biochemical estimations such as glucose, lipid profiles, hepatic marker enzymes, blood metabolites were assayed using biochemistry semi autoanalyzer (Erba chem 5x, Transasia) in the biochemistry research laboratory, Kongunadu Arts and Science College, Coimbatore, India.

## Enzyme linked immunosorbant assay (ELISA) OF AFP, CEA and CA 19.9

The cancer marker enzymes alpha fetoprotein (AEP), cancer embryonic antigen (CEA) and cancer antigen 19.9 (CA 19.9) was analyzed according to the method of [15].

## Assay of mitochondrial enzymes

The mitochondrion of liver tissue was isolated according to the method of [16]. The activities of mitochondrial marker enzymes namely isocitrate dehydrogenase [17], malate dehydrogenase [18] and succinate dehydrogenase [19] is evaluated.

## Assay of lysosomal enzymes

The lysosomal pellet obtained by the method of [20] and used for the estimation of enzymes. To evaluate the activity of lysosomal enzymes namely acid phosphatases (ACP) [21], β- Glucuronidase [22] and cathepsin D [23] are performed.

## Assay of microsomal enzymes

Microsomes can be concentrated and separated from other cellular debris by differential centrifugation based on the method of [24]. The microsomal fraction of liver tissue was used for assaying cytochrome $P_{450}$ reductase [25] and Cytochrome $b_5$ reductase [26].

## Liver and Spleen histopathological studies

For histopathological examination, liver and spleen 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 haematoxylin and eosin (H&E) staining. Pathological changes in liver and spleen tissueses were evaluated under light microscopy.

## Statistical analysis

Results were expressed as mean ± SD of six animals in each group. Statistical significance ($p<0.05$, $p<0.01$) 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

To assess the biochemical activity, the blood glucose, lipid profile and other blood metabolites were analyzed in serum. DEN induced HCC rats (Group II) showed significantly decreased ($p < 0.05$, $p< 0.01$) glucose levels than control rats. Silver nanoparticles of *Solanum villosum* leaves administration significantly increased ($p<0.05$, $p<0.01$, ) the levels of blood glucose when compared to DEN induced groups. It indicates that cancer cells need more energy for survival and cell proliferation. Administration of *Solanum villosum* silver nanoparticles to therapeutic group increased the glucose level as compared to cancer induced group, might be due to the inhibition of cell proliferation in cancer cells, causing decreased utilization of glucose for energy production, indirectly suggesting its chemotherapeutic role in the treatment of HCC. The above results are agreement with [27-28].

The levels of total cholesterol, triglycerides, LDL, and VLDL in serum of control and experimental rats were significantly ($p < 0.05$, $p < 0.01$) increased, and HDL level was decreased in DEN induced HCC rats (table 2). The treatment with SNPs-AESVL showed significantly altered the levels of lipid profile when compared with DEN induced (Group II) HCC rats. Silver nanoparticles of *Solanum villosum* leaves treated rats showed significantly decreased levels of total cholesterol and triglycerides when compared to standard drug treated (Group IV) rats, whereas, significant changes were not observed ($p < 0.05$. $p < 0.01$) in SNPs-AESVL treated rats (Group III) compared to standard drug treated rats (Group IV). [29], who reported that the increased levels of cholesterol and triglycerides were decreased by the administration of the ethanolic root extract of *Operculina turpethum* in the serum of liver cancer induced mice.

The major metabolic vital organ, the liver, plays a key role in cholesterol metabolism in mammals. Abnormal lipid synthesis or defective degradation of lipids is implicated in the pathological condition like cancer [30]. Hence, *Solanum villosum* leaf extract has various functional components, such as flavonoids, alkaloids, phenols and glycosides in the leaves could play an important role in altering body fat and regulating lipid metabolism.

DEN induced cancer rats showed impairment in kidney function which was indicated by the significantly elevated levels of serum urea, creatinine and uric acid. Silver nanoparticles of *Solanum villosum* leaves caused a marked reduction in the levels of urea, creatinine and uric acid in serum exhibiting the effective improvement of kidney function in HCC induced rats. Our findings were similar to that of [31] who showed that induction of oxidative stress by diethylnitrosamine altered the functions of kidney parameters in rats. [32] showed that *Tabernaemontana coronaria* caused a marked reduction in the levels of blood urea and serum creatinine in hepatocellular carcinoma rats.

The hepatic marker enzyme viz, AST, ALT, ALP, LDH, GGT and 5'NT in the serum of control and experimental groups are shown in table 3 respectively. 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. A similar trend was observed in the activities of ALT, ALP, LDH, GGT and 5'NT in serum of cancer bearing animals. Administration of SNPs-AESVL treated rats caused a significant decrease in the levels of serum liver marker enzymes when compared to DEN induced carcinoma rats (Group II). The activities of liver marker enzymes viz AST, ALT, ALP, LDH, GGT and 5'NT in liver of control and experimental rats is presented in table 4. Liver cancer induced rats (Group II) showed significantly ($p < 0.05$, $p < 0.01$) elevated levels of

these enzymes in liver tissues when compared to control rats. Administration SNPs-AESVL treated rats significantly lowered the levels compared to cancer bearing animals. Significant changes were not observed in the silver nanoparticles treated rats (Group III) when compared with the standard drug treated rats.

Our result agrees well with that [33-34] who reported that elevated levels of serum AST, ALT, ALP, LDH and γ-GT, 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. The structural integrity of the cells has been damaged in cancer induced animals, and this results in cytoplasmic leakage of the enzyme into the blood stream, leads to the elevated levels of these enzymes in blood with a subsequent fall in the tissues [35].

Hence, SNPs-AESVL could have exerted their therapeutic effect against DEN induced HCC probably by preventing membrane damage, 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 *Solanum villosum* leaves has the ability to prevent further development of HCC.

Significant decrease in total protein levels in serum was observed in cancer bearing animals (Group II) when compared with control ($p < 0.05$, $p < 0.01$) rats. On the other hand, the levels of total protein were significantly improved ($p < 0.05$, $p < 0.01$) in serum of SNPs-AESVL (Group III) treated rats. The DEN intoxication causes the excessive free radical formation leads to disruption on proteins and thereby reduces the biosynthesis of protein. The treatment of SNPs-AESVL effectively reduces the protein damage caused by DEN may be attributed by the radical scavenging effect and antioxidant activity of *Solanum villosum* leaves.

Hepatoma bearing rats (Group II) possessed increased levels of alpha feto protein, CEA and CA 19.9 when compared to control rats. Treatment with SNPs-AESVL (Group III) decreased the levels of AFP, CEA and CA19.9 when compared to DEN induced HCC rats (Group II). The observed reduction in the levels of AFP, CEA and CA 19.9 in SNPs- AESVL treated animals might be due to a decrease in the rate of tumor development, indicating their anticancer activity of silver nanoparticles of *Solanum villosum* leaves.

The liver mitochondrial enzymes such as, isocitrate dehydrogenase, malate dehydrogenase and succinate dehydrogenase are significantly decreased ($p < 0.05$, $p < 0.01$) in the levels of mitochondrial enzymes in (Group II) DEN induced cancer animals when compared to the control (Group I) animals. Upon administration of SNPs-AESVL (Group III ) animals, these levels were increased comparable to that of cancer bearing (Group II) animals. Increased free radical formation leads to depletion in the activities of ICDH, SDH and MDH enzymes might be due to the increased free radical formation in mitochondria of cancer cells.

The levels of lysosomal marker enzymes ACP, β-Glucuronidase and Cathepsin-D was significantly ($p < 0.05$, $p < 0.01$) increased in DEN induced (Group II) cancer rats when compared to control rats. After administration of SNPs-AESVL treated rats, there was a significant reduction in the levels of lysosomal enzymes when compared to hepatic cancer rats. The presence of flavonoids and other phytochemicals in the leaf extract have the ability to reduce the lysosomal membrane damage. Our findings are in accordance with [36], who reported that after administration of *Rubia cordifolia* root extract.

DEN-induced (Group II) cancer animals exhibited significant increase ($p < 0.05$, $p < 0.01$) in the activities of Cytochrome $P_{450}$ reductase and Cytochrome $b_5$ reductase when compared with (Group I) control animals. Upon SNPs-AESVL treatment (Group III) rats, exhibited a significant decrease in liver tissue when compared to DEN induced rats. *Solanum villosum* leaves are a powerful free radical scavenger which reduces the LPO levels, and decreased the activity of cytochrome P450 reductase and cytochrome b5 reductase in the hepatic tissues. Cytochrome P450 enzymes are widely known for their role in the metabolism of drugs and foreign compounds [37].

Light microscopic observations of silver nanoparticles of *Solanum villosum* leaves treated DEN-induced rat liver is given in Plate 6. 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 (SNPs-EESVL) rats exhibited DEN showed a moderate cancerous change, fatty change, and hydropic degeneration. Groups IV (Standard drug) rats showed fewer neoplastically-transformed cells and the hepatocytes maintained near-normal architecture.

Microscopic observations of silver nanoparticles of *Solanum villosum* leaves in DEN-induced rat spleen are given in Plate 7. The histopathological study of rat spleen showing no congestion and normal architecture in Group I animals. Group II (DEN induced HCC rats) showing multiple numbers of immature megakaryocytes in the white pulps and congestion of red pulps. Group III (SNPs-EESVL treated rats) shows moderate immature megakaryocytes in the white pulps. Group IV Group V (Cyclophosphamide treated rats) shows mild immature megakaryocytes in the white pulps.

The effectiveness of silver nanoparticles obtained via leaf-mediated synthesis is synergistically better in therapeutic activities, when the metal core is capped with biological materials [38]. Metal nanoparticles having a larger surface area which has the ability to carry a relatively high drug dose [39]. Specific targeting of silver nanoparticles towards cancer cells could be due to the differences in pore size and morphology when compared to the normal cells [40].

Silver nanoparticles of *Solanum villosum* leaf extract serves as antitumor agents by decreasing progressive development of tumor cells. This may be due to their inhibitory activities in several signaling cascades responsible for the development and pathogenesis of the disease. The efficient delivery of silver nanoparticles to cancer cells or cancer-associated tissues can be selectively increased by associating the plant extract with silver nanoparticles that are over-expressed on the target cells. Hence, the anticancer property of silver nanoparticles might be attributed by the presence of alkaloids and other phytochemicals of *Solanum villosum* leaf extract which is coated on the silver nanoparticles. The pathways by which AgNPs inhibit the pathway mediating cell proliferation and viability have yet to be explored.

## CONCLUSIONS

The present investigation clearly indicates that SNPs-AESVL were found to be contain biologically active secondary metabolites, which might be responsible for the anticancer properties. Silver nanoparticles of *Solanum villosum* leaf extract may easily enter into the cancer cells, which are having a larger pore size compared to normal cells. Hence, size controlled targeting of silver nanoparticles can prove effective in cancer treatment, might be due to the morphological differences between cancer cells and the normal cells. The validation of *Solanum villosum* leaves has proven to be effective against hepatocellular carcinoma in rats.

**Abbrevations:** SNPs-AESVL, silver nanoparticles of aqueous extract of *Solanum villosum* leaves; ACP, acid phosphatase; ALP, alkaline phosphatase; ALT, Alanine transaminase; AST, aspartate transaminase; EDTA, ethylene diamine tetra acetic acid; HDL, high density lipoprotein; AFP, alfa feto protein; CEA, cancer embryonic antigen; CA 19.9, cancer antigen 19.9.

**Table 1: Effect of Solanum villosum leaves on blood glucose of DEN induced HCC in rats**

| Groups | Glucose (mg / dl) |
|---|---|
| **Group I** | 106.05 ± 2.24 |
| **Group II** | 72.36 ± 1.07 $^{a**}$ |
| **Group III** | 85.40 ± 0.91 $^{b**}$ |
| **Group IV** | 90.58 ± 0.76 $^{c\ ns}$ |

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

Group comparison are made between

Group II vs Group I - a; Group III vs Group II - b; Group IV vs Group III - c. The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non-significance.

## Table 2: Effect of Solanum villosum leaves on lipid profile in serum of DEN induced HCC in rats

| Groups | Total cholesterol (mg / dl) | Triglycerides (mg / dl) | HDL (mg / dl) | LDL (mg / dl) | VLDL (mg / dl) | HDL / LDL |
|---|---|---|---|---|---|---|
| **Group I** | 75.15 ± 1.42 | 68.40 ± 0.64 | 41.36 ± 0.40 | 45.49 ± 0.64 | 14.56 ± 0.74 | 0.90 ± 0.01 |
| **Group II** | 97.48 ± 0.81 $^{a**}$ | 93.58 ± 1.08 $^{a**}$ | 22.53 ± 0.72 $^{a**}$ | 60.56 ± 0.61 $^{a**}$ | 34.30 ± 0.93 $^{a**}$ | 0.37 ± 0.01 $^{a**}$ |
| **Group III** | 77.28 ± 1.01 $^{b**}$ | 78.57 ± 0.99 $^{b**}$ | 34.40 ± 1.20 $^{b**}$ | 50.83 ± 0.98 $^{b**}$ | 18.26 ± 0.52 $^{b**}$ | 0.67 ± 0.02 $^{b**}$ |
| **Group IV** | 78.80 ± 0.58 $^{c\ ns}$ | 79.60 ± 0.57 $^{c\ ns}$ | 32.60 ± 0.76 $^{c\ ns}$ | 49.31 ± 0.67 $^{c\ ns}$ | 19.37 ± 0.49 $^{c\ ns}$ | 0.66 ± 0.01 $^{c\ ns}$ |

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

Group comparison are made between: Group II vs Group I - a; Group III vs Group II - b; Group IV vs Group III - c. The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non-significance.

## Table 3: Effect of Solanum villosum leaves on hepatic marker enzymes in the serum of DEN induced HCC in rats

Values are expressed as mean ± S.D for six animals.
**Group comparison are made between**
Group II vs Group I - a ; Group III vs Group II - b; Group IV vs Group III - c.

| GROUPS | AST | ALT | ALP | LDH | GGT | 5'NT |
|---|---|---|---|---|---|---|
| **Group I** | 91.58 ± 1.17 | 67.16 ± 1.83 | 136.54 ± 1.89 | 387.23 ± 1.62 | 11.63 ± 0.73 | 4.15 ± 0.27 |
| **Group II** | 336.19 ± 2.24 $^{a**}$ | 186.20 ± 1.35 $^{a**}$ | 180.06 ± 1.84 $^{a**}$ | 616.96 ± 3.61 $^{a**}$ | 22.77 ± 0.60 $^{a**}$ | 9.44 ± 0.19 $^{a**}$ |
| **Group III** | 185.56 ± 1.42 $^{b**}$ | 96.75 ± 0.84 $^{b**}$ | 147.50 ± 1.35 $^{b**}$ | 425.20 ± 2.12 $^{b**}$ | 15.47 ± 0.84 $^{b**}$ | 5.68 ± 0.08 $^{b**}$ |
| **Group IV** | 175.83 ± 1.81 $^{c\ ns}$ | 93.15 ± 2.31 $^{c\ ns}$ | 145.00 ± 1.69 $^{c\ ns}$ | 402.26 ± 10.99 $^{c\ ns}$ | 14.35 ± 0.76 $^{c\ ns}$ | 5.80 ± 0.23 $^{c\ ns}$ |

The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non-significance.
**Units:** AST, ALT, LDH - µ moles of pyruvate liberated/L, ALP - µ moles of phenol liberated/L; GGT - µ moles of p – nitroanilide liberated/min/L, 5' NT - µ moles of phosphorus liberated/min/L.

**Table 4: Effect of Solanum villosum leaves on hepatic marker enzymes in the liver of DEN induced HCC in rats**

| GROUPS | AST | ALT | ALP | LDH | GGT | 5'NT |
|---|---|---|---|---|---|---|
| Group I | 72.14 ± 0.94 | 142.77 ± 61.53 | 17.44 ± 0.62 | 358.78 ± 1.84 | 21.28 ± 1.57 | 3.28 ± 0.21 |
| Group II | 41.06 ± 1.53 $^{a**}$ | 129.13 ± 1.56 $^{a**}$ | 32.45 ± 1.32 $^{a**}$ | 432.46 ± 2.06 $^{a**}$ | 36.20 ± 1.27 $^{a**}$ | 7.50 ± 0.14 $^{a**}$ |
| Group IV | 56.16 ± 1.10 $^{b**}$ | 148.61 ± 1.38 $^{b**}$ | 25.94 ± 1.67 $^{b**}$ | 368.83 ± 4.82 $^{b**}$ | 27.23 ± 0.80 $^{b**}$ | 5.25 ± 0.22 $^{b**}$ |
| Group V | 57.40 ± 1.06 $^{c\ ns}$ | 149.30 ± 1.41 $^{c\ ns}$ | 25.35 ± 1.02 $^{c\ ns}$ | 365.90 ± 2.97 $^{c\ ns}$ | 29.87 ± 1.08 $^{c\ ns}$ | 5.49 ± 0.39 $^{c\ ns}$ |

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

**Group comparison are made between**

Group II vs Group I - a ; Group III vs Group II – b; Group IV vs Group III - c

The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non-significance.

**Units:** AST, ALT, LDH - µ moles of pyruvate liberated/min/mg protein, ALP - µ moles of phenol liberated/min/mg protein, GGT - µ moles of p – nitroanilide liberated/min/mg protein, 5' NT - µ moles of phosphorus liberated/min/mg protein.

**Table 5: Effect of Solanum villosum leaves on total protein levels in serum of DEN induced HCC in rats**

| Groups | Total Protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) |
|---|---|---|---|
| **Group I** | 6.45 ± 0.27 | 3.78 ± 0.17 | 2.63 ± 0.30 |
| **Group II** | 4.36 ± 0.36 $^{a**}$ | 2.46 ± 0.30 $^{a**}$ | 1.85 ± 0.08 $^{a**}$ |
| **Group III** | 5.69 ± 0.23 $^{b**}$ | 3.33 ± 0.08 $^{b**}$ | 2.40 ± 0.12 $^{b**}$ |
| **Group IV** | 5.87 ± 0.08 $^{c\ ns}$ | 3.46 ± 0.19 $^{c\ ns}$ | 2.46 ± 0.46 $^{c\ ns}$ |

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

Group comparison are made between: Group II vs Group I - a; Group III vs Group II - b; Group IV vs Group III - c. The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non- significance.

**Table 6: Effect of Solanum villosum leaves on blood metabolites in serum of DEN induced HCC in rats**

| Groups | Urea (mg / dl) | Creatinine (mg / dl) | Uric acid (mg / dl) |
|---|---|---|---|
| **Group I** | 14.87 ± 0.72 | 0.72 ± 0.05 | 1.54 ± 0.14 |
| **Group II** | 28.11 ± 0.58 $^{a**}$ | 0.85 ± 0.01 $^{a**}$ | 2.61 ± 0.23 $^{a**}$ |
| **Group III** | 19.41 ± 0.34 $^{b**}$ | 0.77 ± 0.03 $^{b**}$ | 1.75 ± 0.09 $^{b**}$ |
| **Group IV** | 19.43 ± 0.44 $^{c\ ns}$ | 0.75 ± 0.17 $^{c\ ns}$ | 1.79 ± 0.05 $^{c\ ns}$ |

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

Group comparison are made between: Group II vs Group I - a; Group III vs Group II - b; Group IV vs Group III - c. The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non- significance.

**Table 7: Effect of Solanum villosum leaves on mitochondrial enzymes in liver of DEN induced HCC in rats**

| Groups | ICDH | MDH | SDH |
|---|---|---|---|
| **Group I** | 48.79 ± 1.56 | 29.71 ± 1.35 | 20.87 ± 1.18 |
| **Group II** | 28.27 ± 1.73 $^{a**}$ | 14.85 ± 0.86 $^{a**}$ | 10.20 ± 0.81 $^{a**}$ |
| **Group III** | 43.71 ± 0.54 $^{b**}$ | 24.86 ± 0.76 $^{b**}$ | 17.42 ± 0.66 $^{b**}$ |
| **Group IV** | 44.43 ± 1.11 $^{c ns}$ | 25.37 ± 0.55 $^{c ns}$ | 18.48 ± 0.76 $^{c ns}$ |

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

Group comparison are made between: Group II vs Group I - a; Group III vs Group II - b; Group IV vs Group III - c. The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non-significance. Units: ICDH: nanomoles of α-KG liberated/hr/mg protein; MDH**:** µmoles of NADH oxidized/min/mg protein; SDH**:** nanomoles of succinate oxidized/min/mg protein.

**Table 8: Effect of Solanum villosum leaves on activities of lysosomal marker enzymes in liver of DEN induced HCC in rats**

| Groups | Acid phosphatase | β -Glucuronidase | Cathepsin-D |
|---|---|---|---|
| **Group I** | 5.40 ± 0.25 | 7.84 ± 0.44 | 19.91 ± 0.93 |
| **Group II** | 18.02 ± 0.54 $^{a**}$ | 25.57 ± 0.87 $^{a**}$ | 51.47 ± 1.20 $^{a**}$ |
| **Group III** | 9.96 ± 0.48 $^{b**}$ | 13.52 ± 0.38 $^{b**}$ | 25.12 ± 0.76 $^{b**}$ |
| **Group IV** | 7.99 ± 0.11$^{c*}$ | 10.80 ± 0.15 $^{c*}$ | 22.13 ± 0.21 $^{c*}$ |

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

Group comparison are made between: Group II vs Group I - a; Group III vs Group II - b; Group IV vs Group III - c. The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non-significance. Units: ACP - µmoles of phenol formed/min/mg protein, β-Glucuronidase - µmoles of p-nitrophenol formed/min/ mg protein, Cathepsin D- µmoles of tyrosine liberated hour/mg protein.

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**Table 9: Effect of Solanum villosum leaves on activities of microsomal enzymes in liver of DEN induced HCC in rats**

| Groups | Cytochrome $P_{450}$ reductase | Cytochrome $B_5$ reductase |
|---|---|---|
| **Group I** | 0.84 ± 0.05 | 0.28 ± 0.04 |
| **Group II** | 1.93 ± 0.18 $^{a**}$ | 0.90 ± 0.21 $^{a**}$ |
| **Group III** | 1.62 ± 0.12 $^{b**}$ | 0.79 ± 0.16 $^{b**}$ |
| **Group IV** | 1.68 ± 0.21$^{c\ ns}$ | 0.75 ± 0.17 $^{c\ ns}$ |

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

Group comparison are made between: Group II vs Group I - a; Group III vs Group II - b; Group IV vs Group III - c. The letters a*, b* and c* represents the statistical significance at $p < 0.05$. The letters a**, b** and c** represents the statistical significance at $p < 0.01$. The letter ns represent the non-significance. Units: Cytochrome $P_{450}$ Reducatse - µmoles of NADPH oxidized/min/mg protein. Cytochrome $b_5$ reducatse - µmoles of NADH oxidized FeCN/min/mg protein.

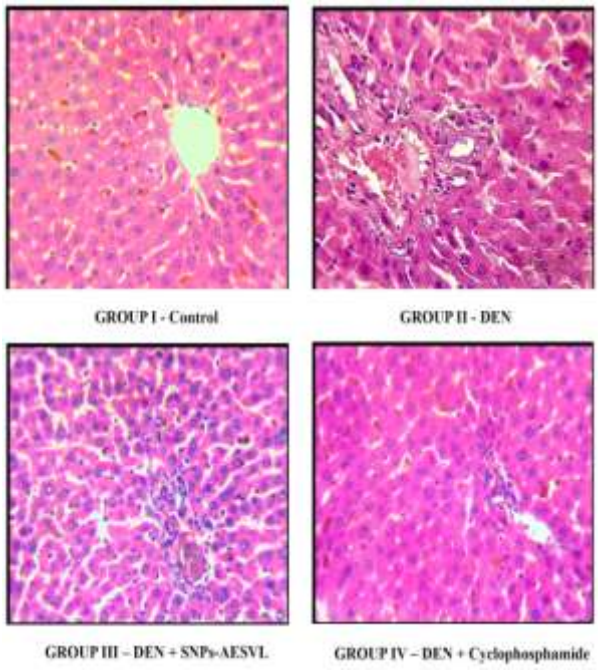


**Fig. (a) Liver histopathology**

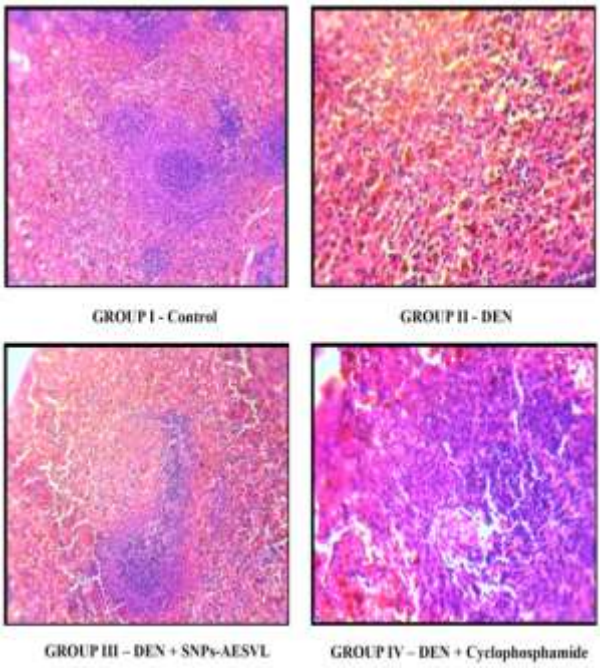


**Fig. (b) Spleen histopathology**

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## Conflict of interest

The authors report no conflict of interest.

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