EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 12
365-373
Year: 2017

# PROTECTIVE ROLE OF SOLANUM NIGRUM LINN LEAF METHANOL EXTRACT ON MALE WISTAR ALBINO RATS AGAINST N-NITROSODIETHYL AMINE INDUCED HEPATOCARCINOMA

**Pandurangan Ayyanar[1], Rajenderan Murugan[2] and Natesan Manoharan[1*]**

[1]Department of Marine Science, Bharathidasan University, Tiruchirappalli, India – 620024.
[2]Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, India 600025.

***Corresponding Author: Natesan Manoharan**
C Department of Marine Science, Bharathidasan University, Tiruchirappalli, India – 620024.

**Article Received on 23/09/2017** **Article Revised on 14/10/2017** **Article Accepted on 04/11/2017**

## ABSTRACT
The protective role of *Solanum nigrum* Linn. was studied in N-nitrosodiethyl amine induced hepatocarcinoma on Wistar albino male rats. The haematological content RBC count, WBC count, Platelet count, PCV, Hemoglobin and ESR were studied and results showed significant (P≤0.001) increased production was recorded. In addition to that liver markers enzyme serum glutamate pyruvate transaminase, serum glutamate oxaloacetic transaminase, acid phophatase, alkaline phophatase, lactate dehydrogenase and γ-glutamyl transpeptidse, and serum biochemical parameters, bilirubin, creatinine, urea, uric acid and serum protein were analyzed. The lipid peroxidation (LPO) and lipid profile were also estimated along with histopathological study in liver tissue. All the parameters are statistically calculated at P≤0.001, P≤0.01and P≤0.05. The recorded results showed significant revert changes in experimental liver tissue.

**KEYWORDS:** Hepatocarcinoma, Solanum nigrum, N-nitrosodiethyl amine.

## INTRODUCTION
Solanacae members are highly valuable for the mouth ulcer and gasterointestinal disorders from the ancient period. Among thus, *Solanum nigrum* Linn. has potential activity was proved scientifically for various health disorders in human like skin diseases, rheumatism, antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic activity (Jain, et al., 2011); ripe fruits inhibit growth and apoptosis in breast cancer cells (Son, et al., 2003), whole plant extract inhibit hepatoma cell death through autophagy and apoptosis (Lin, et al., 2007; Ji, et al., 2008). Steroidal glycoalkaloid and glycoprotein of S. nigrum L., exert cytotoxic and apoptotic activity on human cancer cells (Hu, et al., 1999; Lee, et al., 2004). The leaf extract of this plant showed anticancer, antioxidant (Al-Qirim, et al., 2008), neuroprotective (Jainu, et al., 2005), antimicrobial, antipyretic and liver diseases (Ikeda, et al., 1992; Jamil, et al., 2007). In the present investigation we were tried to ascertain the protective activity of leaf aqueous extract of S. nigrum N-nitrosodiethyl amine induced liver cancer.

## MATERIAL AND METHODS
1. Collection and processing of experimental plants.
The fresh leaves of the *Solanum nigrum* Linn. was collected from Poonamallee, Chennai, Tamil Nadu, Southern India 2009 and brought to the laboratory. The leaves were dried under shade for ten days; they were powdered with blender and sieved through muslin cloth to get uniform particle size of 2 µm. The leaves were stored in labelled moisture free containers for further experimental work.

2. Extraction of phytochemical compounds from *Solanum nigrum.*
The extract obtained with organic solvent methanol in the ratio of 1:3 (wt./vol.) the leaf powder was soaked in solvent and the flask was corked to prevent evaporation and kept under shaking condition (ca. 100 rpm) for 10 days. The crude extracts were filtered through muslin cloth and centrifuged at 3000 rpm for 5 min to remove plant debris. The extract was concentrated using rotary evaporator under reduced pressure and used for the *in vitro* and *in vivo* studies.

3. *In vivo* studies on effect of *Solanum nigrum* leaf extract on hepatocarcinoma induced male Wistar rats
The experimental study were explore the protective efficient of *S. nigrum* leaf extract on DEN induced HCC on Wistar albino male rats. Various biochemical parameters were studied and presented following.

**Experimental animals**

The animal model experiments were conducted according to the ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India and by Animal Ethics Committee Guidelines of University of Madras. male Wistar albino rats weighing ca. 150-180 g were chosen as the experimental animals for the present study. This experiment was conducted for a period of 21.4 weeks. Toxicological evaluation was carried out with various concentrations (100 mg to 1000 mg) of methanolic leaf extract of *S. nigrum* and the treatment dose was fixed at 350 mg/kg body wt. Induction of hepatocarcinoma and treatment with leaf extract of *S. nigrum*. The experimental animals were categorized according to into the following five groups; each group containing six rats.

At the end of the experimental period i.e. after five months, the rats were individually weighed. The animals were anesthetized and the blood was collected by cervical decapitation method. The blood samples collected in centrifuge tubes were kept in inclined position in order to allow complete clotting and then centrifuged at 2500 rpm for 30 min. The clear supernatant was pipetted out and preserved in sterile vials at -20ºC for the biochemical studies. Immediately the rat liver was dissected using sterile surgical scissors and washed with cold phosphate buffered saline (pH 7.0) to remove the blood. The washed liver organ was weighed, photographed and stored in ice cold phosphate buffer (0.1 M, pH 7) for studying various liver marker enzymes and antioxidants. The tissue blotted on clean tissue paper to dryness. From this, 100 mg was taken and homogenized with Tris-HCl buffer (0.1 M, pH 7.4) at 4°C. The homogenates were centrifuged at 2500 rpm for 30 min. The supernatant was transferred sterile vials and stored at -20°C for further biochemical analysis. A peace of liver tissue were preserved in formal saline and used for histopathological studies. All the assays were carried out within 48 hrs after sample collection.

**Estimation of biochemical parameters**

The estimation of hematological contents were estimated by following methods; haemoglobin (Hb) (Drabkin and Austin, 1932), red blood corpuscles (RBC) (Huxtable, 1990), white blood corpuscles (WBC) (Raguramulu *et al*. (1983), packed cell volume (PCV) (Wintrobe, 1932), erythrocyte sedimentation rate (ESR) (Botiger and Svedberg, 1967) and Platelet count (Brecher, 1964).

**ESTIMATION OF VARIOUS BIOCHEMICAL CONSTITUENTS OF SERUM**

The serum biochemical parameters were estimated using standard methods as follows. serum and liver tissue total protein (Lowry *et al*., 1951), Serum bilirubin (Malloy and Evelyn, 1937). Urea (Natelson *et al*., 1951), Uric acid (Caraway, 1963), Serum glutamate pyruvate transminase–(Alanine transaminase, SGPT) (King, 1965a), Serum glutamate oxaloacetate transaminase-Aspartate transaminase (SGOT) (King, 1965a), Acid phosphatase (Acd P) (King, 1965 b), Alkaline phosphatase (Alk P) (King, 1965 b), Lactate dehydrogenase (LDH) (King, 1965c) and γ-Glutamyl transpeptidase (GGPT) (Indirani and Hill, 1977).

**QUANTIFICATION OF BIOCHEMICAL CONSTITUENTS FROM LIVER HOMOGENATE**

Experimental rats liver tissue were homogenised phosphate buffer (pH7.0) and used for estimations of Total liver protein (Lowry *et al.*, 1951), Triglycerides (Rice, 1970), Free cholesterol (Zlatkis *et al.*,1955) and total Cholesterol, ester Cholesterol (Parekh and Jusy, 1970) and Lipid peroxidation (Okhawa *et al*., 1979). The histopathology was taken for all experimental liver tissue to ascertaining hepatoprotective activity of *S. nigrum.*

**Statistical analysis**

The data were subjected for one-way analysis of variance (ANOVA) followed by calculating least significant difference (LSD) test with SPSS/10.0 software. *P* values of less than 0.001, 0.01 and 0.05 were considered to be significant difference between treatments. All values are expressed as mean ± Standard deviation.

**RESULTS**

**Body and liver weight**

In control (GI) and *Solanum nigrum* alone treated group (GV) showed increased body weight and DEN induced (GII) showed drastic reduce body weight than the all experimental group. The post and pretreated group body weight showed increased body weight than the induced group (Table 1).

Liver weight of DEN induced group showed higher than the entire experimental group. Group III and Group IV showed reduced liver weight while compared with induced group. Drug alone group showed more or equal to control group liver weight (Table 1).

**Haemoglobin (Hb)**

In DEN induced hepatocarcinogenesis group showed significantly ($P \leq 0.001$) decreased level than the control and SN extract alone treated group. The SN extract post and pre-treated hepatocarcinogenesis groups showed significantly ($P \leq 0.001$) increased level than the DEN induced group (Tale 2).

**Red blood corpuscles (RBC)**

The RBC production in SN extract post and pre-treated groups showed significant ($P \leq 0.001$) increasing activity than the DEN induced hepatocarcinogenesis group. A significant decreasing RBC production ($P \leq 0.001$) was recorded in DEN induced group while compared with control group and the SN extract alone treated group showed slightly increasing RBC production (Table 2).

**White blood corpuscles (WBC)**

A significant reduction of WBC ($P \leq 0.001$) in DEN induced hepatocarcinogenesis when compared with control and SN extract alone treated groups. The post

and pre-treated groups showed ($P \leq 0.001$) significant increasing WBC production for throughout the experiment while compared with DEN induced hepatocarcinogenesis group (Table 2).

**Packed cell volume (PCV)**
The PCV was significantly ($P \leq 0.001$) reduced in DEN induced hepatocarcinogenesis group than the control group. Both the SN extract treated group showed good ($P \leq 0.001$) increasing PCV than the DEN induced hepatocarcinogenesis group (Table 2).

**Platelet count**
A markedly ($P \leq 0.001$) decreasing count in DEN induced hepatocarcinogenesis group than the control and SN extract alone treated group. The SN pre-treated hepatocarcinogenesis group showed significantly ($P \leq 0.001$) increasing the platelet count than the post-treated experimental group. The post-treated group showed significant ($P \leq 0.05$) increasing count than the DEN induced group (Table 2).

**Erythrocyte sedimentation rate (ESR)**
The ESR rate was highly increased ($P \leq 0.001$) in DEN induced hepatocarcinogenesis group than the entire experimental group. The SN post treated hepatocarcinogenesis group showed significantly ($P \leq 0.001$) decreasing rate than the DEN induced group and it was slightly higher than the SN pre-treated group (Table 2).

**Serum total protein**
The serum total protein significantly increased activity ($P \leq 0.001$) was observed in DEN induced hepatocarcinogenesis group than the all the experimental group. A significant ($P \leq 0.001$) reduction activity was recorded in SN post treated and pre treated hepatocarcinogenesis group while compared with DEN induced group. SN extract alone treated group showed not significant activity (Table 3).

**Serum bilirubin**
Serum bilirubin significantly ($P \leq 0.001$) increased in DEN induced hepatocarcinogenesis group than the control and SN extract alone group. The SN post and pre-treated hepatocarcinogenesis group showed significantly ($P \leq 0.001$) decreasing activity than the DEN induced hepatocarcinogenesis group and the SN extract pretreated group showed significantly ($P \leq 0.001$) near to the control group (Table 3).

**Creatinine**
SN leaf extract post and pretreated hepatocarcinogenesis group showed significant decrease than the DEN induced hepatocarcinogenesis group (2.92 mg/dl). The SN leaf extract alone treated group showed lower creatinine than the control group (1.31 mg/dl) and DEN induced group showed significantly ($P \leq 0.001$) higher than the control group (Table 3).

**Urea**
The content of serum urea was drastically increased in DEN induced hepatocarcinogenesis ($P \leq 0.001$) group than the control group. The post treated group showed significantly ($P \leq 0.001$) lower than the DEN induced group. In SN pretreated group showed significantly lower content of urea than the post treated group. A markedly reduced content of urea was recorded in SN extract lone treated group than the control group (Table 3).

**Uric acid**
DEN induced hepatocarcinogenesis group showed significant increase ($P \leq 0.001$) of uric acid (2.33 mg/dl) than the control group (5.32 mg/dl) (Table 5**).** Both, SN leaf extract post and pretreated hepatocarcinogenesis groups showed significantly decreased level ($P \leq 0.001$) of uric acid when compared with DEN induced hepatocarcinogenesis group. The SN leaf extract alone treated group showed lower level than the control group (Table 3).

**Serum glutamate pyruvate transminase**
DEN induced group showed significant (416.16 U/L; $P \leq 0.001$) increasing level of SGPT than the control and SN leaf extract alone treated group. The significant reduction of SGPT was recorded in SN leaf post (201.3 U/L) and pretreated (167.4 U/L) hepatocarcinogenesis than the DEN induced hepatocarcinogenesis group (Table 4).

**Serum glutamate oxaloacetate transaminase**
The DEN induced hepatocarcinogenesis group showed significant increase ($P \leq 0.001$) of SGOT (75.5 U/L) when compared with control and SN extract alone treated group. The SN extract post and pretreated hepatocarcinogenesis group showed significant ($P \leq 0.001$) decrease level of SGOT (42.1 and 43.2 U/L) than the DEN induced group (Table 4).

**Acid phosphatase**
A significant ($P \leq 0.001$) decreased level of acid phosphatase was recorded in both post and pretreated hepatocarcinogenesis group of SN leaf extract when compared with DEN induced hepatocarcinogenesis group. The SN leaf extract alone treated group showed significantly closed to each other. The DEN induced group showed significant (37.5 U/L; $P \leq 0.001$) increased level than the control group (Table 4).

**Alkaline phosphatase**
DEN induced hepatocarcinogenesis group showed the significant (427.5 U/L; $P \leq 0.001$) increased level of alkaline phosphatase while compared with control group (202.2 U/L). The SN leaf extract treated group showed decreased level enzyme activity than the DEN induced group. In SN leaf extract alone treated group showed markedly decrease level of enzyme activity than the control group (Table 4).

**Lactate dehydrogenase**

LDH enzyme activity level significantly increased in DEN induced hepatocarcinogenesis (P≤0.001; 713.5U/L) group when compared with control (316.2) group and as well as SN extract (321.22 U/L) alone treated group (Table 4). The post (P≤0.05) and pretreated (P≤0.05) group showed significant difference between them and showed decreased level than the DEN induced group.

**γ-Glutamyl transpeptidase**

A significant (P≤0.001) increased level of GGPT enzyme recorded in DEN induced (6.28 U/L) group when compared with control group (Table 4). Both, post and pretreated group of SN leaf extract treated group showed significant decrease than the DEN induced hepatocarcinogenesis group.

**Tissue Total liver protein**

The total liver tissue protein significantly (P≤0.001) reduced in DEN induced hepatocarcinogenesis group (89.4 mg/g liver tissue) when compared with control and SN leaf extract alone treated group. The increased level of tissue protein was recorded in both post (186.6 and 195 mg/g liver tissue) and pretreated hepatocarcinogenesis groups (Table 5).

**Triglycerides**

Triglycerides level in DEN induced group increased significantly (89.40 mg/dl) than the control group (Table 6**).** Both SN leaf extract post and pretreated hepatocarcinogenesis groups showed decrease in triglycerides than the DEN induced hepatocarcinogenesis rat group (P≤0.001) (Table 5). The SN leaf extract alone treated group showed lower level of triglycerides when compared to control group.

**Free cholesterol**

The level of free cholesterol was in DEN induced hepatocarcinogenesis group was significantly higher (78.45 mg/dl) than the control group (21.20 mg/dl). Post (37.24 mg/dl) and pretreated (24.17 mg/dl) hepatocarcinogenesis group showed significant decrease than the DEN induced group (Table 5). The SN leaf extract alone administrated group showed lower level of free cholesterol (20.23 mg/dl) than the control group.

**Total Cholesterol**

The total cholesterol level in DEN induced hepatocarcinogenesis group increased significantly when compared to the control group (P≤0.001, Table 6). The SN leaf extract posttreated group showed lower level (71.42 mg/dl) than the DEN induced group. The pretreated rat showed lower level of total cholesterol than the SN leaf extract posttreated group. The SN leaf extract alone treated showed significant decrease when compared to control group (Table 5).

**Ester Cholesterol**

DEN induced hepatocarcinogenesis group showed (44.3 mg/dl) which was reduced than the control group (41.2 mg/dl; Table 6). Both, SN leaf extract post and pretreated hepatocarcinogenesis groups showed significantly lower level (P≤0.01) of ester cholesterol than the DEN induced hepatocarcinogenesis group (Table 5).

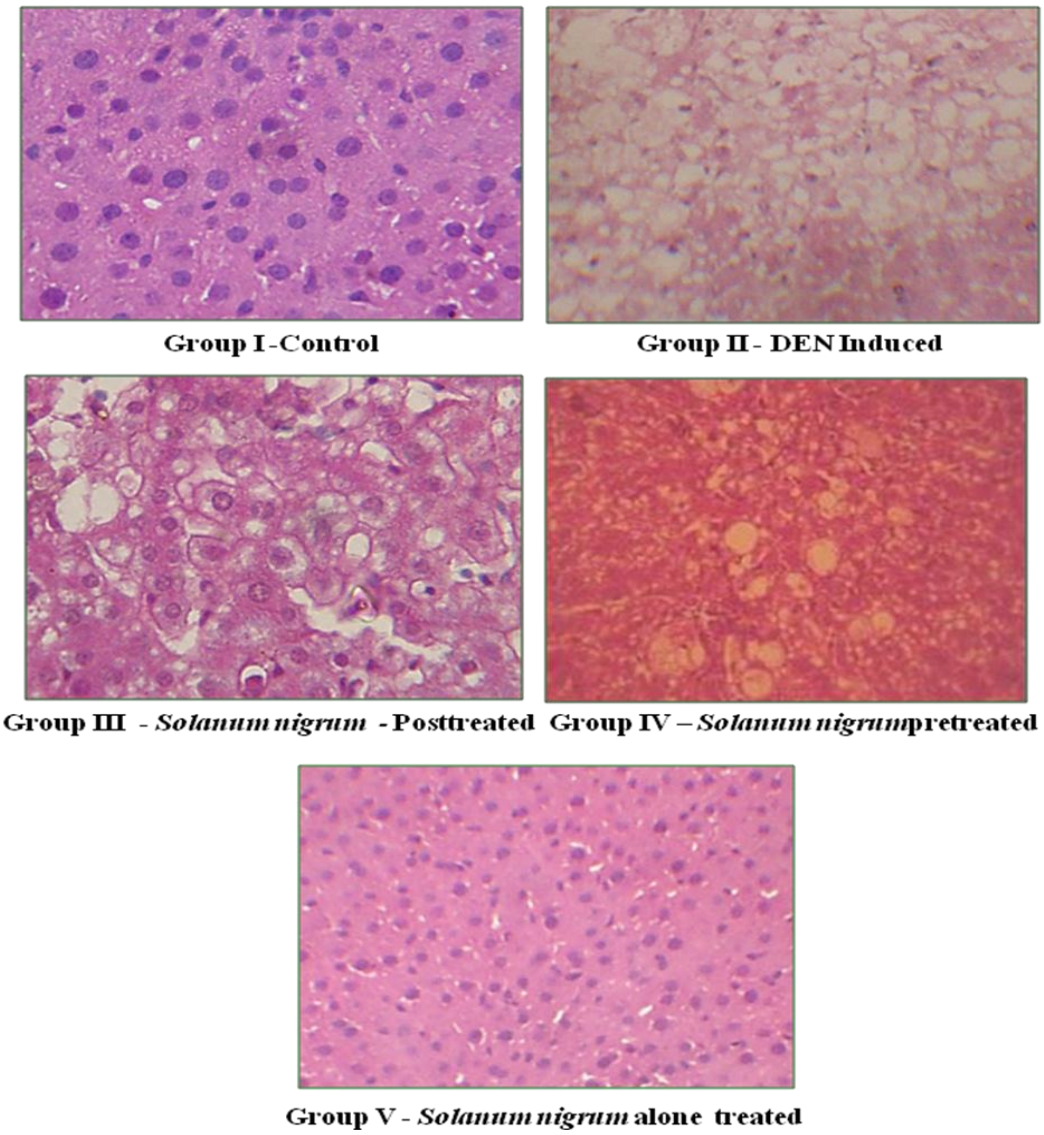
**Lipid peroxidation**

Lipid peroxidation level showed significantly (P≤0.001) increased in DEN induced group than the control group **(**Table 5**).** SN leaf extract post and pretreated group showed significant decrease (*P*≤0.001) in lipid peroxidation when compared to DEN induced group.

**Histopatholgy**

The control liver tissue showed normal hepatocytes with clear architecture of the cell. In DEN induced liver tissue section showed very clear malignant cell along with apoptotic bodies in nucleus with blebing of cell membrane and necrotic region. The SN leaf extract post treated group showed micro vesicle with fatty deposition and damaged cell membrane. Pretreated group liver tissue section showed reverted changes like less condensed nuclear material, less number of apoptotic bodies, reduced size of malignant cell. The SN extract alone treated group does not show any changes and it was similar structure of control group.

**Histopathological variation in experimental animal groups**



Group I-Control

Group II- DEN Induced

Group III - *Solanum nigrum* - Posttreated

Group IV – *Solanum nigrum* pretreated

Group V - *Solanum nigrum* alone treated

**Table 1: Body and liver weight of experimental groups.**

| Experimental Parameter's | Group I Control | Group II Induced | Group III SN Post-treated | Group IV SN Pretreated | Group V Drug alone |
|---|---|---|---|---|---|
| Initial body weight (g) | 186 | 190 | 195 | 175 | 195 |
| Final body weight (g) | 262 | 124 | 176 | 150 | 259 |
| Liver weight (g) | 6.07 | 16.40 | 10.63 | 8.24 | 6.2 |

**Table 2. Variation in hematological characteristics among experimental groups**

| Experimental Parameter's | Group I Control | Group II Induced a | Group III SN Post-treated b | Group IV SN Pretreated c | Group V Drug alone d |
|---|---|---|---|---|---|
| RBC Count | 8.65±0.52 | 3.52±0.49* | 5.97±0.51* | 7.34±0.58* | 8.73±0.59$^{\#}$ |
| WBC Count | 8.31±0.57 | 4.9±0.44* | 5.58±0.35* | 6.93±0.41* | 7.56±0.60$^{\#}$ |
| Hemoglobin | 12.5±1.08 | 5.4±0.66* | 6.1±0.61* | 9.5±0.76* | 13.9±0.55$^{\#}$ |
| Platelets count | 17.2±1.5 | 8.5±2.3* | 12.4±1.3*** | 15.6±1.4* | 17.2±1.2$^{\#}$ |
| Packed Cell Volume | 61.5±3.8 | 29.7±2.9* | 39.6±3.4* | 42.3±4.0* | 51.2±3.1$^{\#}$ |
| Erythrocyte Sedimentation Rate | 3.3±0.25 | 9.25±0.73* | 5.4±0.33* | 4.1±0.35* | 3.6±0.32$^{\#}$ |

**Red blood corpuscles -** millions/mm$^3$ of blood**; White blood corpuscles** millions/mm$^3$ of blood**; Hemoglobin:** g/dl; **Platelet:** millions/mm$^3$ of blood; **Packed cell volume -** %**; Erythrocyte sedimentation rate -** mm/h.

*P*≤0.001 = * : a – Compared with Group I – Control; b compared with Group I and a; c compared with a and b; d compared with Group.
*P*≤0.01 = ** : a – Compared with Group I – Control; b compared with Group I and a; c compared with a and b; d compared with Group.
*P*≤0.05 = *** : a – Compared with Group I – Control; b compared with Group I and a; c compared with a and b; d compared with Group
# not significant with control.

**Table 3: Variation in serum biochemical characteristics among experimental groups.**

| Experimental Parameter's | Group I Control | Group II Induced a | Group III SN Post Treated b | Group IV SN Pretreated c | Group V Drug alone d |
|---|---|---|---|---|---|
| Serum Total Protein (mg/dl) | 12.1±0.75 | 24.18±1.87* | 16.24±1.2* | 18.49±1.4* | 12.7±0.68# |
| Total Bilirubuin (mg/dl) | 0.78±0.05 | 2.71±0.14* | 1.31±0.07* | 0.82±0.06* | 0.70±0.05# |
| Creatinine (mg/dl) | 1.31±0.09 | 2.92±0.15* | 1.35±0.10* | 1.21±0.11* | 1.05±0.08# |
| Urea (mg/dl) | 14.07±1.36 | 28.2±2.14* | 17.25±1.75* | 16.4±1.5* | 12.4±2.08# |
| Uric acid (mg/dl) | 2.33±0.17 | 5.32±0.25 * | 3.78±0.24* | 3.05±0.25* | 2.0±0.22# |

**Table 4: Variation in serum biochemical characteristics among experimental groups.**

| Experimental Parameter | Group I Control | Group II Induced a | Group III SN Post Treated b | Group IV SN Pretreated c | Group V Drug alone d |
|---|---|---|---|---|---|
| SGOT (U/L) | 153.7±13.5 | 416.16±11.04* | 201.3±20.8* | 167.4±18.2* | 159.2±15.6# |
| SGPT(U/L) | 41.3±2.6 | 75.5±5.3* | 45.3±3.7* | 42.1±3.3* | 43.2±3.1# |
| Alk P+ (U/L) | 202.2±19.6 | 427.5±45.8* | 241.3±20.7* | 211.01±19.8* | 197.25±16.5# |
| Acd P+(U/L) | 15.6±11.3 | 37.5±3.21* | 19.5±5.41* | 18.2±1.49* | 16.2±1.21# |
| LDH (U/L) | 316.2±24.56 | 713.5±56.01* | 349.64±30.2*** | 323.13±28.9*** | 321.22±23.7# |
| GGPT(U/L) | 2.91±0.1 | 6.28±0.5* | 3.6±0.3* | 3.6±0.2* | 3.1±0.1# |

*P*≤0.001 = * : a – Compared with Group I – Control; b compared with Group I and a; c compared with a and b; d compared with Group.
*P*≤0.01 = ** : a – Compared with Group I – Control; b compared with Group I and a; c compared with a and b; d compared with Group.
*P*≤0.05 = *** : a – Compared with Group I – Control; b compared with Group I and a; c compared with a and b; d compared with Group.
# not significant with control.

**Table 5: Liver tissue biochemical level in experimental groups.**

| Experimental Parameter's | Group I Control | Group II Induced a | Group III SN Post Treated b | Group IV SN Pretreated c | Group VDrug alone d |
|---|---|---|---|---|---|
| Tissue total protein | 213.1±15.6 | 89.4±8.05* | 186.6±13.9* | 195.1±14.6* | 221.3±16.4# |
| Total Cholesterol | 59.21±8.3 | 128.22±4.9* | 71.42±5.4* | 65.31±4.6* | 55.35±4.09# |
| Triglycerides | 43.16±3.8 | 89.40±9.8* | 56.03±5.1* | 53.34±4.57* | 41.02±3.56# |
| Free cholesterol | 21.20±1.6 | 78.45±4.4* | 37.24±2.1* | 24.17±1.8* | 20.23±1.5# |
| Ester cholesterol | 41.2±3.1 | 44.3±1.9* | 38.5±2.3* | 34.2±2.4* | 42.20±3.2# |
| LPO | 2.21±0.17 | 5.34±0.28* | 3.91±0.11* | 2.98±0.12* | 2.16±0.16# |

Tissue total protein : (mg/g tissue of liver); Total Cholesterol - mg/gm of liver tissue; Triglycerides - mg/gm of liver tissue; Free cholesterol - µg/g dry defatted tissue; Cholesteryl ester - µg/g dry defatted tissue; LPO : η moles of melondialdehyde/mg protein).

## DISCUSSION

Drug discovery from medicinal plants has played an important role in the treatment of cancer and indeed most new applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman *et al.*, 2000, 2003; Butler, 2004). Chemoprevention strategies target each of carcinogenesis steps including antiinitiation strategies

(ex. DNA repair, detoxification, free radical scavenging and carcinogen metabolism) and antipromotion and strategies (ex. Free radical scavenging, progression suppression, differentiation induction, immunity enhancement, inflammation reduction, increase in apoptosis, altered gene expression and decrease in angiogenesis (Greenwald 2002; Tsao, *et al.,* 2004). N-diethylnitrosamine is converted to active electrophilic species following hydroxylation, resulting in the formation of unstable hydroxyalkyl compounds that are subsequently converted to alkyl carbonium ions (Williams and Weisburger, 1991). DEN toxicity is primarily associated to an excessive production of free radicals in the liver. As a consequence, reactive electrophilic intermediates are formed, which overwhelms the antioxidant defences and ultimately proceeds to oxidative stress paving way to liver damage (Kang *et al.,* 2007). 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-radical oxidizing species are regularly produced in animals treated with carcinogens, and also in human tissues (Sun 1990). ROS formed from endogeneous (or) exogeneous sources are highly reactive, toxic and mutagenic (Halliwell 1994). DEN has been shown to generate free radicals (Halliwell and Gutteridge, 1989), an uncompromised free radical generation in the liver overwhelms the antioxidant status and ultimately proceeds to oxidative stress paving way to carcinogenesis (Gey 1993).

Lipid peroxidation is one of the major mechanisms of cellular injury caused by free radicals (Esterbauer and Chesseman, 1990). Administration of DEN has been reported to generate lipid peroxidation products like MDA and 4-hydroxy nonenal that may interact with various molecules leading to oxidative stress and carcinogenesis (Hietanen, *et al*., 1987). The level of LPO increases with the administration of DEN during hepatocarcinogenesis. This dynamic action may further lead to uncompromised production of free radicals overwhelming the cellular antioxidant defence (Klaunig and Kamendulis, 2004). It has been extensively reported that free radicals participated in DEN-induced hepatocarcinogenesis (Laughton, *et al.,* 1991). The cellular pro-oxidant states are due to the increased concentrations of ROS, organic peroxides and radicals, which are suggested to be involved in pathological processes leading to the development of cancer (Cerutti, 1985). In our study the recorded results showed LPO is highly increased in DEN induced hepatocarcinogenesis group and it was reverted in SN extract post and pretreated group. Among the SN treated group the pretreated group showed lower melondialdehyde production. The prolonged treatment of SN extract alone treated group does not show any increment for throughout the experiment. Liver damage caused by DEN generally reflects instability of liver cell metabolism which leads to distinctive changes in the serum enzyme activities (Plaa and Hewitt, 1989). Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Mitra *et al.,* 1998). Serum transaminases, SGOT, SGPT, LDH and GGTP are representative of liver function; their increased levels are indicators of liver damage. The elevation of SGPT activity is repeatedly credited to hepatocellular damage and is usually accompanied by a rise in SGPT (Plaa and Hewitt, 1989). Increase in ALP reflects the pathological alteration in biliary flow. The discharge of LDH reflects a non-specific alteration in the plasma membrane integrity and/or permeability. GGPT is an enzyme embedded in the hepatocyte plasma membrane, mainly in the canalicular domain; again the liberation of this enzyme into serum indicates damage to the cell and thus injury to the liver. It is important to point out that serum GGPT activity is considered to be one of the best indicators of liver damage (Bulle, *et al.,* 1990).

With regards to plasma enzyme determinations, an increase in SGPT, SGOT, AcP, AlkP, LDH and GGTP was detected in DEN induced group. Ordinarily, liver cell damage is characterized by a rise in serum enzymes (Brautbar and Williams, 2002; Rajesh and Latha, 2004). Generally, SGOT concentrations are consistently higher than SGPT levels which are expected since body cells contain more SGOT than SGPT (Mayne, 1996). Therefore, SGOT appears in higher concentrations in a number of tissues (liver, kidneys, heart and pancreas) and is released slowly in comparison to SGPT. But since SGPT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than SGOT and within limits can provide a quantitative assessment of the degree of damage sustained by the liver (Al Mammary *et al.,* 2002). The SGOT, SGPT and alkaline phosphatase was significantly increased in CCl4 induced hepatotoxicity rats and Hb was significantly decreased in that treatement (Elhag et al, 2011, Raju et al., 2003, Atanu 2011). Hence, the treatment *Solanum nigrum* methanolic leaf extract showed a significant decrease of liver marker and increasing content of Hb and the observed results showed plant extract protect the hepatotoxicity. In the present investigation also, the level of SGOT, SGPT, ALP was decreased and Hb increased in SN extract treated groups and as well as reduction of ESR is indicate the plant extract stimulate the immune system. Therefore, the serum creatinine, urea and uric acid also reduced in hepatocarcinogenesis treated group with SN leaf extract while compared with the DEN induced group results and this results proved the SN leaf extract influence to regulate the biochemical cycle in lever cell. In our recorded results also showed highly elevated level of SGPT, SGOT, AcP, AlkP, LDH and GGTP in the DEN induced hepatocarcinoma group than the control, post and pretreated group of SN leaf extract and SN alone treated group. And all the liver marker enzymes were reduced in post ant pre treated groups.

The lipid profile of the experimental group showed decreased in *Solanum nigrum* leaf extract treated groups than the DEN induced hepatocarcinogenesis group. The reduction of liver marker enzymes in hepatocarcinogenesis group is indicating the protection of cell integrity and revert the damaged cells to normal cell. It due to the presence of polyphenolic compounds with active principle which is protect the cell environment in SN leaf extract. The results presented in this study indicate that the hepatocarcinogenesis induced by DEN is effectively protected by pretreatment administration of the methanolic extract of *S. nigrum* leaf extract showed better efficacy towards DEN-induced liver injury when compared with post-treatment. Our results highlight the capacity of the extract to modulate the levels of LPO in DEN induced hepatocellular carcinogenesis and this result is shows the cell membrane rejuvenation or repairing efficient principle present in SN leaf extract. The prolonged period administration of SN leaf extract alone group does not shows any adverse effect. From the results obtained, we suggest that extract may be developed as an effective chemotherapeutic agent for protecting the liver diseases.

**ACKNOWLEDGEMENT**

The authors is highly thankful to Prof. R. Rengasamy, Director, Centre for Advanced Studies in Botany, University of Madras, Chennai, India 600025 for providing the necessary infrastructure to conduct the study and our heartfelt thanks to Dr. Vijay Lakshmi, Vishnavi Histopathology and Microphotographs, Chennai, India for taking the histopathology and microphotographs.

**REFERENCES**

1. Al Mammary, M., Al-Habori, M., Al-Aghbari, A.M. and Baker, M.M. 2002. Investigation into the toxicological effects of *Catha edulis* leaves: a short-term study in animals. *Phytotherapy Res*, 16: 127–132.
2. Al-Qirim T, Syed M, Moyad S, Ghassan S and Naheed B. Effect of Solanum nigrum on immobilization stress induced antioxidant defense changes in rat. Research journal of Biological Sciences, 2008; 3: 1426–9.
3. Botiger, L.E. and Svedberg, C.A. 1967. Normal erythrocyte sedimentation rate and age. *Br. Med. J*, 2: 85.
4. Brautbar, N. and Williams II, J., 2002. Industrial solvents and solvent and liver toxicity: risk assessment, risk factors and mechanisms: review. *Inter. J. Hyg. Environ. Health*, 205: 479–491.
5. Brecher, G., Jakobek, E.F., Schneiderman, M.A., Williams, G.Z. and Schmidt, P.J. 1962. Size distribution of erythrocytes. *Ann. N. Y. Acad. Sci*, 99: 242.
6. Bulle, F., Mavier, P., Zafrani, E.S., Preaux, A.M., Lescs, M.C., Siegrist, S., Dhumeaux, D. and Guellaen, G. 1990. Mechanism of γ-glutamyl transpeptidase release in serum during intrahepatic and extrahepatic cholestasis in the rat: a histochemical, biochemical and molecular approach. *Hepatol*, 11: 545– 550.
7. Caraway, W.T. 1963. Uric acid. *In*: *Standard method of clinical chemistry*, D. Seligon, ed. Academic press, New York, 4: 239-247.
8. Cerutti, P.A. 1985. Prooxidant states and tumor promotion. *Science*, 227: 375–381.
9. Drabkin, D.L. and Austin, J.H. 1992. Spectrophotometric studies, spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. *J. Biol. Chem*, 98: 719-733.
10. Esterbauer, H. and Chesseman, K.H. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxy-nonenal. *Meth. Enzymol*, 186: 407–421.
11. F. O. Atanu1*, U. G. Ebiloma1 and E. I. Ajayi2. 2011. A review of the pharmacological aspects of *Solanum nigrum* Linn. Biotechnology and Molecular Biology Review, 6(1): 001-007.
12. Gey, K.F. 1993. Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. *Br. Med. Bull*, 49: 679–699.
13. Greenwald, P. Cancer chemoprevention. *Brit. Med. J*, 324: 714–718.
14. Halliwell, B. 1994. Free radical, antioxidant and human disease: curiosity, cause or consequence?. *Lancet*, 344: 721–724.
15. Halliwell, B. and Gutteridge, J.M.C. 1989. Free Radicals in Biology and Medicine, 2nd ed., Clarendon Press, Oxford, UK, 543.
16. Hietanen, E. Ahotupa, M. and Bartsch, H. 1987. Lipid peroxidation and chemically induced cancer in rats fed lipid rich diet. *In*: K. Lapis, S. Kcharst (Eds.), In Carcinogensis and Tumor Progression, vol. 4, Akademiaikiado, Budapest, 9-16.
17. Hu K, Kobayashi H, Dong A, Jing Y, Iwasaki S and Yao X, Antineoplastic agents. III. Steroidal glycosides from Solanum nigrum. Planta Med, 1999; 65: 35–38.
18. Huxtable, R. J. 1990. Activation and pulmonary toxicity of pyrrolizadine alkaloids. *Pharmacol. Therap*, 47: 371-390.
19. Ikeda T, Tsumagari H and Nohara T. Steroidal oligoglycosides from Solanum nigrum growing in Azerbaijan. Biologicheskie Nauki, 1992; 3: 15–8.
20. Indirani, N. and Hill, P.G. 1977. Partial purification and some properties of γ-glutamyl transpeptidase from human bile. *Biochem. Biophys. Acta*, 179: 588-590.
21. Jain R, Sharma A, Gupta S, Sarethy IP, Gabrani R"Solanum nigrum: current perspectives on therapeutic properties." Altern Med Rev, 2011 Mar; 16(1): 78-85
22. Jainu M, Devi CSS. Antiulcerogenic and ulcer healing effects of Solanum nigrum (L.) on experimental ulcer models: Possible mechanism for the inhibition of acid formation. Journal of

Labooratory and Clinical Medicine, 2005; 98: 417-27.
23. Jamil A, Shahid M, Khan MMH and Ashraf M. Screening of some medicinal plants for isolation of antifungal proteins and peptides. Pakistan Journal of Botany, 2007; 39(1): 211-221.
24. Ji YB, Gao SY, Ji CF and Zou X, Induction of apoptosis in HepG2 cells by solanine and Bcl-2 protein. J Ethnopharmacol, 2008; 115: 194–202.
25. Kang, J., Wanibuchi, H., Morimura, K., Gonzalez F. and Fukushima, S. 2007. Role of CYP2E1 in diethylnitrosamine-induced hepatocarcinogenesis *in vivo*. *Cancer Res*, 67: 11141–11146.
26. King, J. 1965b. The transferases – alanine and aspartate transaminases. *In*: Practical clinical enzymology, D. Van (ed). Nostrand Company Ltd, London, 121-138.
27. King, J. 1965c. The dehydragenase or oxidoreductases–lactate dehydragenase. *In*: Practical clinical enzymology, D. Van (ed). Nostrand Company Ltd, London, 83-93.
28. Klaunig, J.E. and Kamendulis, L.M. 2004. The role of oxidative stress in carcinogenesis. *Ann. Rev. Pharmacol. Toxicol*, 44: 239–267.
29. Laughton, M.J., Evans, P.J., Moroney, M.A., Hoult, J.R. and Halliwell, B. 1991. Inhibition of mammalian 5-lipoxygenase and cycloxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem. Pharmacol*, 42: 1673–1681.
30. Lee SJ, Oh PS, Ko JH, Lim KandLim KT,A150-kDaglycoprotein isolated from Solanum nigrum L. has cytotoxic and apoptotic effects by inhibiting the effects of protein kinase C alpha, nuclear factor-kappa B and inducible nitric oxide in HCT-116 cells. Cancer Chemother.
31. Lin HM, Tseng HC, Wang CJ, Chyau CC, Liao KK, Peng PL, et al, Induction of autophagy and apoptosis by the extract of Solanum nigrum Linn in Hep G2 cells. J Agric Food Chem, 2007; 55: 3620–3628.
32. Lowry, O.H., Rosebrough, N.H., Farr, A.L. and Randall, R.I. 1951. Protein measurement with the Folin-Phenol reagent. *J. Biol. Chem*, 193: 265-275.
33. Malloy, H.T. and Evelyn, K.A. 1937. The determination of bilirubin with the photometric calorimeter. *J. Biol. Chem*, 119: 481-490.
34. Mayne, P.D. 1996. Clinical Chemistry in Diagnosis and treatment, 6th ed. Arnold London/Oxford University Press Inc., New York.
35. Mitra, S.K., Venkataranganna, M.V., Sundaram, R. and Gopumadhavan, S. 1998. Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. *J. Ethnopharmacol*, 63:181–186.
36. Natelson, S., Scott, M.L. and Beffa, D. 1951. A rapid method for the estimation of urea in biological fluids by means of the reaction between diacetyl and urea. *Amer. J. Chem. Pathol*, 21: 275-281.
37. Newman, D.J., Cragg G.M. and Snader, K.M. 2003. Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod*, 66: 1022–1037.
38. Newman, D.J., Cragg, G.M. and Snader, K.M. 2000. The influence of natural products upon drug discovery. *Nat. Prod. Rep*, 17: 215–234.
39. Okhawa, H., Ohisshi, N. and Yagi, K. 1979. Assay of lipid peroxides in animal tissues by thiobarbutric acid reaction. *Ann. Biochem*, 95: 351-358.
40. Parekh, A.C. and Jung, D.H. 1970. Cholesterol determination with ferric acetateurayl acetate and sulphuric acid-ferrous sulphate reagents. *Anal. Chem*, 42: 1423-1427.
41. Pharmacol, 2004; 54: 562–572.
42. Plaa, G.L. and Hewitt, W.R. 1989. Detection and evaluation of chemically induced liver injury, *In*: A. Wallace Hayes (Ed.), Principles and Methods of Toxicology, Raven Press, New York, 399–628.
43. R. A. M. Elhag1, S. M. A. El Badwi2, A. O. Bakhiet3* and M. Galal1. 2011. Hepatoprotective activity of Solanum nigrum extracts on chemically induced liver damage in rats. Journal of Veterinary Medicine and Animal Health, August 2011; 3(4): 45-50.
44. Raguramulu, N., Nair, K.M. and Sundaram, S. K. 1983. *In*: A manual of laboratory techniques, Silver Prints, Hydrabad, India, 254.
45. Rajesh, M.G. and Latha, M.S. 2004. Preliminary evaluation of the antihepatotoxic effect of Kamilari, a polyherbal formulation. *J. Ethnopharm*, 91: 99–104.
46. Raju K, Anbuganapathi G, Gokulakrishnan V, Rajkapoor B, Jayakar B, Manian S. Effect of dried fruits of Solanum nigrum LINN against CCl4-induced hepatic damage in rats. Biol. Pharm. Bull, 2003; 26(11): 1618-1619.
47. Rice, E. 1970. Triglycerides in serum. Standard Methods. *Clinical Chem*, 6: 215.
48. Son YO, Kim J, Lim JC, Chung Y, Chung GH and Lee JC, Ripe fruit of Solanum nigrum L. inhibits cell growth and induces apoptosis in MCF-7 cells. Food Chem Toxicol, 2003; 41: 1421–1428.
49. Sun, Y. 1990. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radic. Biol. Med*, 8: 583–599.
50. Tsao, A.S., Kim, E.S. and Hong, W.K. 2004. Chemoprevention of cancer. *CA-A Cancer J. Clinicians*, 54: 150–180.
51. Williams, G.M. and Weisburger. J.H.1991. Chemical carcinogenesis. *In*: *Casarett and Doull's Toxicology: The basic science of poisons.* Eds.: M.O. Amdur, J. Doull and C.D. Klassen. Pergamon Press, New York, 127–200.
52. Wintrobe, M.M. 1932. The size and haemoglobin content of the erythrocyte; Methods of determination and clinical application. *J. Lab. Clin. Med*, 17: 899.
53. Zlatkis, B., Zak A.J. and Boyle. 1953. Now method for direct determination of serum cholesterol. *J. Lab. Clin. Med*, 41: 486-492.