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Hepatoprotective and Antioxidant activity of *Scoparia dulcis* Linn, against N-Nitrosodiethylamine (DEN) induced Hepatotoxicity in experimental Rats

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Abstract

Scoparia dulcis Linn, belongs to the family Scrophulariaceae and have speculated Medicinal properties. In this present investigation, the antioxidant and hepatoprotective activity of the aqueous extracts of *Scoparia dulcis* was evaluated against N-nitrosodiethylamine (DEN) induced liver cirrhosis in experimental rats. In group III hepatotoxicity induced animals, an oral dose of 500 mg/kg, of the aqueous extracts of *Scoparia dulcis* exhibited a significant (P<0.01) decrease in marker enzyme levels and increased levels of antioxidant enzymes. There is a significant reduction in the LPO levels in the group III extract treated animals against DEN induced liver toxicity. From the results of our investigation, we concluded that, the aqueous extract of *Scoparia dulcis* L showed significant antioxidant defence mechanism and hepatoprotective activity.

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INTRODUCTION

Hepatotoxicity is an injury to the liver associated with its dysfunction caused by exposure to

various drugs and toxicants. The hepatotoxicant may be divided into genotoxic and non-genotoxic. Genotoxicants directly interact with DNA, forming covalent adducts and induces genetic changes upon cell replication. Non-genotoxic chemicals stimulate tumour formation by altering kinetics of cell proliferation, cell death and cell differentiation through a variety of epigenetic pathways.[1] Exposure to an agent that causes induction of an enzyme important in the biotransformation of a drug, may led to increased formation of its reactive metabolites and greater risk of toxicity.

N-nitosodiethylamine (DEN) is a potent hepatotoxin and if co-administered with promoters like phenol-barbital, it also causes hepatocarcinogenesis. [2, 3, 4] DEN, a polycyclic-aromatic hydrocarbon is a common environmental pollutant which is produced by the incomplete combustion of organic materials, fossil fuels, cigarette smoke, residential heating units, power plants and in certain occupational settings. [5, 6] It is reported that generation of reactive oxygen species (ROS) by DEN causes by-products of cellular metabolism that have direct effect on cell development, growth and survival. [7]

Medicinal plants, since time immemorial have been in use for treatment of various diseases all over the world. [8] *Scoparia dulcis* L. (SD) belongs to the Family: Scrophulariaceae is a glabrous under shrub commonly found in the tropical and sub-tropical regions. Phytochemical screening has shown that *Scoparia dulcis* contains diterpenoids, flavonoids, [9] tannins, alkaloids, triterpenes, hexacosonol, β-sitosterol, ketone, dulcitone and amellin, an antidiabetic compound. [10] The plant of *S. dulcis* is known to possess antidiabetic activity, [11] antiulcer activities. [12] An aqueous extract of *S. dulcis* showed marked *in vitro* antioxidant activity, which supports the therapeutic effects claimed by traditional practitioners. [13] An active and safe drug is needed for the treatment of liver toxicity and hepatitis. [14] The active principles present in *Scoparia dulcis* have been shown to exhibit cytotoxic and antitumor activity. [15] The present study was undertaken to conclude the hepatoprotective effect of the ethanolic extract of *S. dulcis* in DEN induced experimental rats.

MATERIALS AND METHODS

Preparation of Plant Extract

Scoparia dulcis L. whole plants were collected during the month of January from Chidambaram, Tamilnadu, India. The plant was authenticated by Chief Botanist, Captain Srinivasa Multi Drug Research Institute for Ayurveda and Siddha (CCRAS). A voucher specimen has been deposited at the department of pharmacology and Environmental Toxicology, Dr.ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, Tamilnadu, India. The whole fresh plant was dried under shade at room temperature for seven days and then reduced to a coarse powder. This powder was used for the preparation of aqueous extracts. The extract obtained was filtered and concentrated to a viscous consistency at 55°C (the yield was 12.6 % w/w for aqueous extracts).

Animals

Healthy adult Wistar albino rats of both sexes weighing between 110±20g were used for the present study. They were obtained from the Central Animal House Facility, Dr.ALMPGIBMS, Taramani, University of Madras, Chennai (IAEC No: 07/012/08). The animals were kept in polypropylene cages and received standardized rat pellet and water *ad libitum*. All the procedures were done in compliance with the guidelines issued by the Institutional Animal Ethics Committee.

Chemicals

DEN was purchased from Sigma Chemical Company, St Louis, MO, U.S.A. All other Chemical including solvents used were of high purity and of analytical

grade marketed by Glaxo Laboratories, Mumbai and Sisco Research Laboratories Pvt. Ltd, Mumbai, India.

DEN induced Hepatotoxicity in animal models

Laboratory animals treated with low and intermediate doses of DEN (50 and 100 mg/kg), light and moderate liver necrosis has been documented with or without associated inflammatory process. In the animals treated with the highest dose of DEN (200mg/kg) extensive liver necrosis has been observed with associated inflammatory process. [16] Therefore it was decided to use DEN for inducing hepatotoxicity in the present investigation. In the present investigation rats were divided into four groups of six animals each. Group I: Control animals were administered with normal saline (0.9%). Group II:Animals received a single intra-peritoneal injection of DEN at a dose of 200mg/kg in normal saline to induce hepatotoxicity. Group III: After inducing toxicity, animals were administered with the aqueous extract of *Scoparia dulcis* L. orally at a concentration of 500mg/kg body weight for a period of 28 successive days. Group IV: Animals received only plant extract orally at a concentration of 500mg/kg body weight for a period of 28 successive days. At the end of the experiment, all the animals were sacrificed by cervical decapitation. Blood and liver samples were collected to for the estimate of various biochemical parameters.

Biochemical Estimations

Estimation of Nucleic Acids

Estimation of Deoxyribonucleic acid (DNA) DNA was estimated by the method of [17]. Estimation of Ribonucleic acid (RNA) RNA was estimated by the method of [18].

Estimation of Marker Enzymes

AST and ALT by the method of [19], ALP and ACP was assayed by the method of [20] as described by Balasubramanian *et al.,* [21], 5'- Nucleotidase was assayed by the method of [22], Gamma glutamyl transpeptidase was estimated by the method of [23], LDH was assayed by the method of [24].

Estimation of Macromolecular Damages

Assay of lipid peroxidation (LPO)

The level of lipid peroxides was assayed by the method of [25], Peroxide induced tissue lipid peroxidation, Ascorbate and ferrous sulphate induced tissue lipid peroxidation was assayed by the method of [26].

Estimation of Enzymic Antioxidants

The activity of superoxide dismutase (SOD) and catalase (CAT) was determined by the method of [28], The activity of glutathione peroxidase (GPx) was assayed by the method of [29].

Estimation of Non-Enzymic Antioxidants

Glutathione was estimated by the method of [30], the level of ascorbic acid was estimated by the method of [31], and the level of vitamin E was estimated by the method of [32].

Statistical Analysis

Data are presented as the mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups of by using SPSS 12.5 student's versions. Comparisons were made between group II and IV with group I and III for animal studies. $P<0.01$ was considerable statistically significant in all cases.

RESULTS

Table 1show the levels of nucleic acids (DNA and RNA) in liver of control and experimental animals. In group II animals, the levels of nucleic acids were significantly decreased due to DEN induction ($p<0.01$). These were significantly increased in drug

treated group III animals ($p<0.01$). However, no significant changes were observed in group IV drug control animals when compared to the control group I animals.

Table 1: The levels of Nucleic acids in liver of control and experimental animals

Parameters (mg/g wet tissue)	Group I Control	Group II DEN	Group III DEN + Limonin	Group IV Limonin
DNA	6.79±0.19	2.38±0.18[a]	4.49±0.17[a,b]	6.68±0.18[b,c]
RNA	5.70±0.17	3.40±0.20[a]	4.47±0.18[a,b]	5.84±0.18[b,c]

Values are expressed as mean ± SD for six animals in each group
a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV, c - Group III Vs Group IV The significance at the level of $p<0.01$

Table 2 shows the activities of marker enzymes such as AST, ALT, ACP, ALP, LDH, 5´-ND and γ-GT levels in serum of control and experimental animals. Significantly increased levels of marker enzymes were noticed in serum of DEN induced hepatotoxicity animals, when compared with group I control animals ($p<0.01$). The levels of marker enzymes were found to be decreased in *Scoparia dulcis* L. treated group III animals when compared with group II animals ($p<0.01$). No significant changes in marker enzymes activity were observed in group IV only extract administrated animals when compared to group I control animals.

Table 2: Effect of *Scoparia dulcis* L. on the activities of marker enzyme in serum of control and experimental animals

Parameters	Group I Control	Group II DEN	Group III DEN + Limonin	Group IV Limonin
ALT (μ moles of pyruvate liberated / mg protein/min)	31.60±2.29	40.53±2.65[a]	32.48±2.40[a,b]	30.50±2.12[b,c]
AST (μ moles of pyruvate liberated / mg protein/min)	6.15±0.25	9.10±0.59[a]	7.20±0.31[a,b]	6.08±0.34[b,c]
ALP (μ moles of phenol liberated / mg protein/min)	180.66±11.09	280.55±19.38[a]	191.45±11.80[a,b]	179.69±12.73[b,c]
ACP (μ moles of phenol liberated / mg protein/min)	39.36±2.25	49.81±2.99[a]	41.65±2.68[a,b]	39.00±2.28[b,c]
γ-GT (n moles of p-nitro aniline formed / mg protein/min)	3.47±0.18	7.32±0.36[a]	4.92±0.16[a,]	3.39±0.14[b,c]
LDH (μ moles of pyruvate liberated / mg protein/min)	1.40±0.28	2.89±0.52[a]	1.70±0.10[b]	1.39±0.07[b]
5´-ND (n moles of phosphate liberated / mg protein/min)	3.90±0.27	7.30±0.24[a]	4.10±0.29[a,b]	3.60±0.23[b,c]

Values are expressed as mean ± SD for six animals in each group
a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV, c - Group III Vs Group IV The significance at the level of $p<0.01$

Table 3 represents the activities of marker enzymes such as AST, ALT, ACP, ALP, LDH, 5´-ND and γ-GT levels in liver of control and experimental animals. The activities of marker enzymes were decreased in Group II toxicity bearing animals ($p<0.01$). On the contrary, in *Scoparia dulcis* L. treated Group III animals, the levels of marker enzymes were reversed to near normal when compared to group II animals ($p<0.01$). However, there were no significant changes were observed in Group IV aqueous extract of *Scoparia dulcis* L. administrated animals when compared with Group I control animals.

Table 3: Effect of *Scoparia dulcis* L. on the activities of marker enzyme in liver of control and experimental animals

Parameters	Group I Control	Group II DEN	Group III DEN + Limonin	Group IV Limonin
ALT (μ moles of pyruvate liberated/mg protein/min)	10.44±0.54	7.39±0.36[a]	9.50±0.45[a,b]	10.30±0.66[b,c]
AST (μ moles of pyruvate liberated/mg protein/min)	7.33±0.32	5.51±0.33[a]	6.35±0.31[a,b]	7.29±0.27[b,c]
ALP (μ moles of phenol liberated/mg protein/min)	13.71±0.57	7.58±0.78[a]	11.85±0.53[a,b]	13.50±0.57[b,c]
ACP (μ moles of phenol liberated/mg protein/min)	6.66±0.18	4.18±0.34[a]	5.69±0.23[a,b]	6.90±0.11[b,c]
γ-GT (n moles of p-nitro aniline formed/mg protein/min)	8.15±0.46	5.10±0.23[a]	7.55±0.28[a,b]	8.06±0.28[b,c]
LDH (μ moles of pyruvate liberated/mg protein/min)	3.21±0.19	1.60±0.10[a]	2.98±0.16[a,b]	3.19±0.10[b,c]
5´-ND (n moles of phosphate liberated/mg protein/min)	5.35±0.18	3.30±0.18[a]	4.90±0.21[a,b]	5.40±0.18[b,c]

Values are expressed as mean ± SD for six animals in each group
a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV, c - Group III Vs Group IV The significance at the level of $p<0.01$

Table 4 & 5 shows the effect of *Scoparia dulcis* L. on Lipid peroxidation in the serum & liver of control and experimental animals respectively. In serum and liver, the basal, hydrogen peroxide, ascorbate and ferrous sulphate induced LPO were significantly increased in group II toxicity induced animals ($p<0.01$) when compared with control group I animals. Conversely, the administration of *Scoparia dulcis* L. significantly reduces the peroxidation reaction in group III drug treated animals ($p<0.01$). However, no marked alterations of lipid peroxidation were observed in group IV drug control animals when compared to the group I control animals.

Table 4: The levels of lipid peroxidation in serum of control and experimental animals

Parameters (n moles of TBARS formed/mg protein/min)	Group I Control	Group II DEN	Group III DEN + Limonin	Group IV Limonin
Basal	1.80±0.19	3.37±0.18[a]	2.47±0.17[a,b]	1.72±0.18[b,c]
H_2O_2 induced	4.65±0.17	10.45±0.20[a]	6.47±0.18[a,b]	4.60±0.18[b,c]
Ascorbic acid induced	5.34±0.14	10.40±0.19[a]	7.62±0.16[a,b]	5.19±0.23[b,c]
$FeSO_4$ induced	5.05±0.46	9.71±0.16[a]	7.15±0.22[a,b]	4.±99.17[b,c]

Values are expressed as mean ± SD for six animals in each group
a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV, c - Group III Vs Group IV
The significance at the level of $p<0.01$

Table 5: The levels of lipid peroxidation in liver of control and experimental animals

Parameters (n moles of TBARS formed/mg protein/min)	Group I Control	Group II DEN	Group III DEN + Limonin	Group IV Limonin
Basal	1.37±0.17	3.36±0.18[a]	2.07±0.22[a,b]	1.31±0.16[b,c]
H_2O_2 induced	2.82±0.21	4.02±0.26[a]	3.57±0.18[a,b]	2.78±0.16[b,c]
Ascorbic acid induced	1.94±0.17	3.93±0.16[a]	2.60±0.18[a,b]	1.89±0.16[b,c]
$FeSO_4$ induced	2.13±0.20	4.61±0.18[a]	3.50±0.17[a,b]	2.05±0.19[b,c]

Values are expressed as mean ± SD for six animals in each group
a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV,
c - Group III Vs Group IV The significance at the level of $p<0.01$

Table 6 shows the activities of Enzymic and Non-enzymic antioxidants in the serum of control and experimental animals. In group II DEN induced animals, the activities of enzymic antioxidants SOD, CAT, GPx and non-enzymic antioxidants GSH, Vit-C and Vit-E were significantly decreased as compared to that of group I control animals ($p<0.01$). These enzymes levels were increased significantly reverted back to near normal in drug treated group III animals when compared with group II animals ($p<0.01$). No remarkable changes were observed in group IV drug control animals when compared with group I control animals.

Table 7 shows the activities of superoxide dismutase, catalase, glutathione peroxidise, glutathione, ascorbic acid and α-tocopherol in the liver of control and experimental animals. A significant decrease in the level of these enzymes were observed in the DEN induced group II animals ($p<0.01$) when compared to group I control animals. Interestingly, a significant increase in the level of these antioxidant enzymes was observed in the *Scoparia dulcis* L. treated group III animals ($p<0.01$). No remarkable changes were observed in group IV drug control animals when compared with group I control animals.

Table 6: Effect of *Scoparia dulcis* L. on Enzymic and Non-enzymic antioxidants in serum of control and experimental animals

Parameters	Group I Control	Group II DEN	Group III DEN + Limonin	Group IV Limonin
Super Oxide dismutuse (units/mg protein/min)	4.90±0.15	3.28±0.18[a]	4.75±0.16[a,b]	5.10±0.12[b,c]
Catalase (µ mol of H_2O_2 consumed/mg protein/min)	27.18±0.50	19.22±52	25.66±81	28.10±21
Glutathione peroxidase (µg of GSH utilized/mg protein/min)	2.99±1.66	1.26±1.91[a]	2.10±1.82[a,b]	3.05±0.68[b,c]
Glutathione reduced (µg/mg protein /min)	5.18±0.21	3.32±0.10[a]	4.11±0.17[a,b]	5.51±0.30[b,c]
Vitamin C (mg/g of wet tissue)	2.15±0.03	1.40±0.10[a]	1.95±0.07[a,b]	2.55±0.08[b,c]
Vitamin E (mg/g of wet tissue)	1.80±0.19	0.94±0.22[a]	1.65±0.14[a,b]	2.13±0.19[b,c]

Values are expressed as mean ± SD for six animals in each group
a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV, c - Group III Vs Group IV The significance at the level of $p<0.01$

Table 7: Effect of *Scoparia dulcis* L. on Enzymic and Non-enzymic antioxidants in liver of control and experimental animals

Parameters	Group I Control	Group II DEN	Group III DEN + Limonin	Group IV Limonin
Super Oxide dismutuse (units/mg protein/min)	8.15±0.12	4.59±0.15[a]	7.30±0.19[a,b]	8.28±0.13[b,c]
Catalase (µ mol of H_2O_2 consumed/mg protein/min)	72.85±1.88	35.10±1.82[a]	60.10±1.85[a,b]	73.0±1.58[b,c]
Glutathione peroxidase (µg of GSH utilized/mg protein/min)	75.40±0.22	53.19±0.15[a]	71.65±0.11[a,b]	75.91±0.12[b,c]
Glutathione reduced (µg/mg protein /min)	4.51±0.20	2.80±0.12[a]	3.15±0.19[a,b]	4.70±0.30[b,c]
Vitamin C (mg/g of wet tissue)	1.60±0.04	0.50±0.08[a]	0.99±0.10[a,b]	1.77±0.02[b,c]
Vitamin E (mg/g of wet tissue)	5.36±2.08	3.14±0.15[a]	4.39±0.84[a,b]	5.64±0.60[b,c]

Values are expressed as mean ± SD for six animals in each group
a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV, c - Group III Vs Group IV The significance at the level of $p<0.01$

DISCUSSION

Hepatotoxicity refers to liver dysfunction as a result of an overload of toxic chemicals or drugs in the body. DEN is reported to generate reactive oxygen species (ROS) that leads to an oxidative stress. It is well established that protein, lipids and DNA are the prime targets of oxidative injury. [33] The interruption in uracil nucleotide dependent biosynthesis of nucleic acid results in DNA damage. The decrease in the levels of DNA and RNA due to chemical injury to liver has been reported earlier by [34] and [35]. The observed decrease in both DNA and RNA levels in DEN induced animals may be due to its inhibitory effects on the DNA synthesis and RNA transcription in hepatocytes and lead to the damage of the plasma membrane. Treatment with *Scoparia dulcis* L. aqueous extract probably prevented DEN induced UTP depletion and a subsequent suppression of nucleic acids by restricting the formation of toxic metabolites from DEN.

The liver functions as a filter that separates out harmful substances from the bloodstream. When there is an excess of chemicals filtering through the liver, the overload is more than the liver can process and leads to hepatotoxicity. The levels of certain enzymes are drastically altered in any kind of liver damage of which transaminases are regarded as the important markers of liver function. These marker enzymes sensitively reflect the status of the liver damage. [36] Along with transaminases ACP, ALP,

LDH, 5´-ND and γ-GT are also sensitive markers of hepatic damage. [37, 38] In the present investigation, the increased levels of marker enzyme in serum and decreased level in the liver are indicators of cellular damage and loss of functional integrity of the cell membrane due to DEN administration. Treatment with aqueous extract *Scoparia dulcis* L. brought back these enzymes to near normal level by probably preserving the functional integrity of the hepatocytes, showing its hepatoprotective action against DEN induced liver damage.

Lipid peroxidation mediated by free radical is considered to be primarily responsible for cell membrane destruction and cell damage. LPO has been implicated in a number of deleterious effects on cells, such as increased membrane rigidity, osmotic fragility, decreased cellular deformation and reduced membrane fluidity. Increase in the levels of TBARS indicates enhanced LPO, leading to tissue injury and failure of the antioxidant defence mechanism. [39] DEN administration resulted in the formation of LPO products and leads to cellular damage in the group II animals. In the present study, *Scoparia dulcis* L. extract administration decreased lipid peroxides level which may be due to the free radical scavenging activity of the plant extract.

Antioxidants acts as radical scavenger and inhibit LPO and other free radical-mediated processes, thereby protecting the human body from various diseases. [40] There is a natural dynamic balance between the output of free radicals generated in the body and the antioxidant defense system that quenches or scavenges them and thereby protecting the body against pathogenesis. [41] The first lines of defence against free radicals are endogenous enzymic antioxidants such as SOD, Cat, GPx and the Second lines of defence are the non-enzymic antioxidants such as glutathione, Vitamin-C and Vitamin-E. [42] DEN administration resulted to uncompromised free radical production overwhelming the cellular defence. In the present investigation, *Scoparia dulcis* L. extract stabilized the normal fluctuations in enzymic and non-enzymic antioxidant levels due to its antioxidant efficacy.

The biochemical alterations observed in hepatotoxicity status seems to be mainly due to an oxy radical mediated mechanism, involving LPO, under conditions of reduced antioxidant defences. *Scoparia dulcis* L. protects against LPO by mechanisms that may be dependent on changes in the antioxidant defence system. These observations indicate that, *Scoparia dulcis* L**.** administration may be protective against DEN induced hepatotoxicity.

CONCLUSION

From our investigation, we concluded that aqueous extracts of *Scoparia dulcis* L**.** possessed important hepatoprotective and antioxidant activity against DEN induced liver toxicity in Wistar albino rats may be due to its antioxidant and free radical scavenging properties. Therefore, number of scientific reports indicated certain flavonoids, alkaloids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties. Presence of those phytochemicals in *Scoparia dulcis* L**.** may be responsible for the protective effect in DEN induced liver damage in experimental rats.

REFERENCE

1) Wogan GN. Impacts of chemicals on liver cancer risk. Semin Cancer Biol 2000; 10: 201-210.
2) Brown JL. N-Nitrosamines. Occup Med 1999; 14: 839-848.
3) Reh BD, Fajen JM. Worker exposure to introsamines in a rubber vehicle sealing plant. Am Ind Hyg Assoc J 1996; 57: 918-923.
4) Sullivan BP, Meyer TJ, Stershic MT, Keefer LK. Acceleration of N-nitrosation reactions by electrophiles. IARC Sci Publ 1991; 105: 370-374.
5) Bostrom CE, Gerde P, Hanberg A, Jernstrom B, Johansson C, Kyrklund T, Rannug A, Tornquist M,

Victorin K, Westerholm R. Cancer risk assessment, indicators and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ. Health. Perspect 2002; 110: 451-488.

6) Lucier G, Hook GER. Dietary mutagens special issue. Environ Health Perspect 1986; 67-72.

7) Parola M, Robino G. Oxidative stress-related molecules and liver fibrosis. J Hepatol 2001; 35(2): 297-306. Review.

8) Satyanarayana K. Chemical examination of *Scoparia dulcis* (Linn). Journal of the Indian Chemical Society 1969; 46: 765-766.

9) Ramesh P, Ramachandran N, Sankara SS. Flavonoids of *Scoparia dulcis* and *Stemodia viscosa*. Current Science 1979; 48: 67.

10) Hayashi T. Bioactive diterpenoids from a tropical herb, *Scoparia dulcis* L. Interactions Congr Ser 1998; 1157: 197-205.

11) Pari L, Latha M. Antidiabetic effect of *Scoparia dulcis*: effect on lipid peroxidation in streptozotocin diabetes. General Physiology Biophysics 2005; 24: 13–26.

12) Melania B, Kristina S, Paul S. Antiulcer activity of water extract of *Scoparia dulcis*. Fitoterapia 2008; 79: 587–588.

13) Ratnasooriya WD, Jayakody JRAC, Premakumara GAS, Ediriweera ERHSS. Antioxidant activity of water extract of *Scoparia dulcis*. Fitoterapia 72005; 6: 220–222.

14) Mukherjee PK, Wahile A, Kumar V, Rai S, Mukherjee K. Marker Profiling for A Few Botanicals Used for Hepatoprotection in Indian System of Medicine. Drug Information J 2006; 40: 131–139.

15) Hayashi K, Lee JB, Maitani Y, Toyooka N, Nemoto H, Toshimitsu H. The role or a HSV thymidine kinase stmulating substance scopadulciol, in improving the efficacy of cancer gene therapy. J Gene Med 2006; 8: 1056-62.

16) Barbisan LF, Miyamoto M, Scolastici C, Salvadori DM, Ribeiro LR, Eira AF, de Camargo JL. Influence of aqueous extract of Agaricus blazei on rat liver toxicity induced by different doses of diethylnitrosamine. J Ethnopharmacol 2002; 83(1-2): 25-32.

17) Burton K. A study of the conditions and mechanisms of diphenylamine reaction for colorimetric estimation of deoxyribonucleic acid. J.Biochem 1956; 62: 315-323.

18) Rawal VM, Patel VS, Rao GN, Desai RR. Chemical and biochemical studies on cataractous human lenses-III- Quantitative study of protein. RNA and DNA. Arogya J. Health Sci 1977; 3: 69-75.

19) King J. The Transferases-alanine and aspartate transaminases. In: Practical and Clinical Enzymology, (Eds.) by: Van Nostrand Co.Ltd, London, 1965a, pp. 121-138.

20) Bergmeyer HO. Methods of enzymatic analysis. (Eds). Academic Press, New York 1963.

21) Balasubramanian MP, Dhandayuthapani S, Nellaiappan K, Ramalingam K. Comparative studies on phosphomonoesterase in helminthes. Helminthologia 1983; 20: 111-120.

22) Luly P, Barnabei O, Tria E. Hormonal control in vitro of plasma membrane bound Na^+, K^+-ATPase of rat liver. Biochemica Biophysica Acta 1972; 282: 447-452.

23) Rosalki SB, Rau D. Serum gamma glutamyl transpeptidase activity in alcoholism. Clin Chem Acta 1972; 39: 41-17.

24) King J. The phosphohydrolysases-acid and alkaline transaminase, In: Practical Clinical Enzymology, (Eds.) by: Van Nostrand Co.Ltd, London, 1965b, pp. 191-208.

25) Hogberg J, Larson RE, Kristoferson A, Orrenius S. NADPH-dependent reductase solubilised from microsomes by peroxidation and its activity. Biochem. Biophys. Res. Commun 1974; 56: 836-842.

26) Devasagayam TPA, Tarachand U. Decreased lipid peroxidation in the rat kidney during gestation. Biochem Biophys Res Commun 1987; 145: 134-138.

27) Markland S, Markland G. Involvement of superoxide anion radical in the autoxidant of pyrogallol and a convenient assay for superoxide dismutase. Er J Biochem 1974; 47: 469-474.

28) Sinha AK. Colorimetric assay of catalase. Anal. Biochem 1972; 47: 389-394.

29) Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical

role as a component of glutathione peroxidase. Science 1973; 179: 588-590.

30) Moron MS, Depierre JW, Mannervik B. Levels of glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochem. Biophys. Acta 1979; 582: 67-78.

31) Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol 1971; 62: 3-11.

32) Desai ID. Vitamin E analysis methods for animal tissues. Methods Enzymol 1984; 105: 138-144.

33) Athar M, Agarwal R, Bickess DR and Mukhar H. In: "Pharmacology of the skin" CRC Press Inc; Boca, USA, 1992, pp. 260.

34) Francavilla A, DiLeo A, Polimeno L, Gavaler J, Pellicci R, Todo S, Kam I, Prelich J, Makowka L, Starzl TE. The effect of hepatic stimulatory substance, isolated from regenerating hepatic cytosol, and 50,000 and 300,000 subfractions in enhancing survival in experimental acute hepatic failure in rats treated with D-galactosamine. Hepatology 1986; 6(6): 1346-51.

35) Kato O, Fukuda Y, Hayakawa T, Izuta S, Yoshida S. Serum DNA polymerase β as a indicator for fetal liver injury of rat induced by D-galactosamine hydrochloride and lipopolysachride. Biochem Biophy Acta 1998; 1380: 369-376.

36) Zimmerman HJ. Hepatotoxicity. The adverse effects of drugs and other chemicals on the liver. Appleton Century Croft. New York. 1978, 91-121.

37) Nan JX, Park EJ, Nam JB, Zhao YZ, Cai XF, Kim YH, Sohn DH, Lee JJ. Effect of Acanthopanax koreanum Nakai (Araliaceae) on D-galactosamine and lipopolysaccharide-induced fulminant hepatitis. J Ethnopharmacol 2004; 92(1): 71-7.

38) Sallie R, Fredger JM, William R. Drugs and liver. Biopharm Drug Disposition 1991; 12: 251-254.

39) Comporti M. Three models of free-radicals induced cell injury. Chem Biol Interact 1989; 72: 1-56.

40) Czinner E, Hagmasi K, Blazovics A, Kery A, Szoke E, Lemberkovics E. The *in vitro* effect of *Helichrysi flos* on microsomal lipid peroxidation. Journal of Ethnopharmacology 2001; 77: 31–35.

41) Kolanjiappan K, Manoharan S, Kayalvizhi M. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clin Chim Acta 2002; 326(1-2):143-9.

42) Liebler DC. The role of metabolism in the antioxidant function of vitamin E. Crit Rev Toxicol 1993; 23(2): 147-69. Review.