



Asian Journal of **Biochemistry**

ISSN 1815-9923



Academic
Journals Inc.

www.academicjournals.com

Anti-carcinogenic Effect of *Solanum trilobatum* in Diethylnitrosamine Induced and Phenobarbital Promoted Hepatocarcinogenesis in Rats

²M.S. Jahan, ¹G. Vani and ¹C.S. Shyamaladevi

¹Department of Biochemistry, University of Madras, Guindy Campus, Chennai-600 025, India

²Department of Biochemistry, Mohamed Sathak College of Arts and Science, Chennai, Tamilnadu, India

Corresponding Author: M.S. Jahan, Department of Biochemistry, Mohamed Sathak College of Arts and Science, Chennai, Tamilnadu, India

ABSTRACT

Aim of the study is found the anticancer effect of ST on chemically induced hepatocellular carcinoma. The methanolic extract of *Solanum trilobatum* (ST) prevented 2 stage carcinogenesis in Wistar rats induced by diethyl nitrosamine (DEN) and promoted with Phenobarbital (PB). Animals were induced for hepatic carcinoma with DEN (200 mg kg⁻¹ b.wt. i.p.), promoted with PB (0.05%) in basal diet for a period of 3 and 6 months. Animals were also co-treated with methanolic extract of ST (250 mg kg⁻¹ b.wt. in DMSO orally) for the same period of time (DEN+PB+ST). Administration of ST significantly decreased the levels of tumor markers-carcinoembryonic antigen (CEA) and α -fetoprotein (AFP) in serum compared to their untreated counterparts and also favorably altered the marker enzymes AST, ALT, ALP, GGT and LDH in both serum and liver and 5'nucleotidase in liver. These results show the antitumor effect of ST against DEN induced hepatocarcinogenesis.

Key words: Beta solamarine, antitumor, carcinoembryonic antigen, N-diethylnitrosamine (DEN), Hepatocarcinogenesis, marker enzymes, *Solanum trilobatum*, sobatum, solasodine

INTRODUCTION

Primary liver cancer ranks fifth in the frequency among all the malignancies in the world with an estimated number of 4, 37, 00 new cases in 1990. The vast majority of primary liver cancer is hepatocellular carcinoma (HCC). Accumulating epidemiological and experimental evidence has revealed the influence of a number of naturally occurring and synthetic compounds on the drug detoxification and HCC incidence. Several groups of plants have been shown to possess anticancer effect against hepatocarcinoma. Among the various plants associated with the life of Indians, one such plant is *Solanum trilobatum* (ST), Linn. (Family: Solanaceae) that is used traditionally in different parts of India for various ailments (Madhavan and Balu, 1999). The partially purified glycoalkaloid of ST contains sobatum that inhibits growth of Dalton's lymphoma (DLA). Ehrlich's Ascites Cells (EAC), L929 cells and inhibits growth of peritoneal and solid tumours induced by DLA and EA cell lines (Mohan and Devi, 1996). Sobatum inhibits DMBA induced papilloma in skin carcinogenesis (Mohan and Devi, 1997a), potentiates protective effect against cyclophosphamide induced toxicity (Mohan and Devi, 1998a), reduces side effects of radiation induced toxicity (Mohan and Devi, 1998b) and does not produce cytogenetic effect in bone marrow erythrocytes in mice (Mohan *et al.*, 1996). The antimutagenic effect of sobatum has also been reported (Mohan and Devi, 1997b). Diethylnitrosamine (DEN) exposure occurs through diet, that is

detectable in edible vegetable oil, alcoholic drinks, steamed and fried fish, formed endogenous in the body also from the use of tobacco products, cosmetics, pharmaceutical products and agricultural chemicals. DEN is one of the most important environmental carcinogens in its class that primarily induces tumours of the liver because of its relatively simple metabolic pathway and potent carcinogenic activity (Leopky, 1994). Hepatocarcinogenesis induced by DEN is a favourite model in rats as it facilitates the study of mechanism of chemical carcinogenesis and response of HCC of anticancer drug therapy. The role solasodine an active constituent of *Solanum trilobatum* on sarcoma and skin cancer has been identified. The inhibitory role of the methanolic extract of *Solanum trilobatum* on DEN and PB induced hepatocarcinogenesis have been reported (Shahjahan *et al.*, 2004, 2005). The modulatory role of the methanolic extract on antioxidant status and drug metabolism during hepatocarcinogenesis have been reported. The combination of cisplatin with *S. trilobatum* could effectively treat the B[a]P-induced lung cancer in mice by offering protection from reactive oxygen species damage and also by suppressing cell proliferation, preliminary study indicates that *S. trilobatum* could be used to promote the health status of fish in intensive finfish aquaculture. This study presents the effect of *Solanum trilobatum* during hepatocarcinogenesis on the levels of tumour markers to favour its use as a therapeutic agent.

MATERIALS AND METHODS

Chemicals: Dimethylsulfoxide (DMSO) was purchased from SD Fine chemical, Chennai, India. Diethylnitrosamine (DEN) was purchased from Sigma, USA. All chemicals and reagents used were of analytical grade.

Plant material: The methanolic extract of *Solanum trilobatum* was gifted by Dr. Sharada Vasanth, Research Officer, Department of Organic Chemistry, Central Research Institute of Ayurvedha, Arumbakkam and Chennai-106.

Preparation of the plant extract: One kilograms of shade dried, coarsely powdered plant material (*Solanum trilobatum*) was charged in an aspirator bottle and allowed to soak in 90% alcohol for 48 h at room temperature. The extract was filtered and concentrated on a water bath to 20 mL. The filtrate was again concentrated in a china dish and dried in vacuum desiccators at 4°C. The yield of the plant extract was about 10%. Since, the yield was less, the procedure was repeated again to get sufficient yield, till the completion of the experimental work in animal models. *Solanum trilobatum* extract (ST) was dissolved in 10% DMSO for oral administration.

Experimental design: Male Wistar albino animals (150-200 g) were obtained from the animal house of Tamil Nadu Veterinary and Animal Sciences, Madhavaram, Chennai. Animals were provided feed and water *ad libitum*. All studies were carried in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC No. 01\047\04).

Animals were divided into 4 groups with 6 animals each group:

- **Group 1:** Control animals were administered orally treated with 1 mL of 10% DMSO for the entire experimental period of 3 and 6 months (CONTROL)
- **Group 2:** Drug control animals were administered orally with 250 mg kg⁻¹ b.wt. day⁻¹ of ST dissolved in 1 mL of 10% DMSO orally, for the entire experimental period of 3 and 6 months (ST)

- **Group 3:** Animals induced for HCC using DEN (200 mg kg⁻¹ bw. single i.p. in normal saline) Phenobarbital (PB) 0.05% in basal diet, for the entire experimental period of 3 and 6 months (DEN+PB)
- **Group 4:** Animals induced for HCC (as Group 3 animals) and co-administered with 250 mg kg⁻¹ b. wt. day⁻¹ of ST extract dissolved in 1 mL of 10% DMSO orally, for the entire experimental period of 3 and 6 months (DEN+PB+ST)

Animals were killed at the end of experimental period by cervical decapitation. Blood was collected and serum was separated. Liver tissues were excised from the animals and homogenate was prepared using 0.01 M Tris-HCl, pH 7.4 buffer.

The levels of tumour markers - carcinoembryonic antigen (CEA) and alpha fetoprotein (AFP) in serum were quantitated on the solid phase enzyme linked immunosorbent assay (ELISA) as described (Macnab *et al.*, 1978).

The activities of marker enzymes - Aspartate aminotransferase (AST) (King, 1965b). Alanine aminotransferase (ALT) (Bergmeyer, 1974) Lactate dehydrogenase (King, 1965b) γ -Glutamyl transpeptidase (King, 1965a) and Alkaline phosphatase (King, 1965c). Were assayed both in the serum and liver tissue and 5' nucleotidase in liver tissue (Luly *et al.*, 1972).

Statistical analysis: Values are presented as mean \pm SD. Student's t test was used for statistical significance (Bailey, 1959).

RESULTS

Significant detectable levels of CEA and AFP were observed in DEN + PB animals, whereas no detectable levels were observed in control animals. However in DEN+PB+ST animals, the levels of both CEA and AFP were significantly decreased ($p < 0.001$) both at the end of 3rd and 6th month compared to DEN+PB animals confirming a positive prognosis on ST administration. No detectable levels of CEA and AFP were observed in serum of ST animals confirms its non-toxic nature (Table 1).

Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH) γ -Glutamyl transpeptidase (GGT) and Alkaline phosphatase (ALP).

The activities of all the marker enzymes showed a significant increase ($p < 0.001$) in DEN+PB animals at the end of 3rd and 6th months compared to control. In DEN+PB+ST animals, the hepatoprotective nature of ST was noted from a significant ($p < 0.001$) decrease in the activities compared to DEN+PB animals. The non-toxic nature of ST was witnessed from the maintenance of these enzyme activities in ST animals (Table 2).

Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH) γ -Glutamyl transpeptidase (GGT) and Alkaline phosphatase (ALP).

Units: AST - μ moles of pyruvate liberated min/mg protein; ALT - μ moles of pyruvate liberated min/mg protein; ALP - μ moles of phenol liberated min/mg protein; LDH - μ moles of pyruvate formed min/mg protein; GGT - μ moles of p-nitroaniline formed min/mg protein; 5' Nucleotidase - nmoles of inorganic phosphorus/min/mg protein.

A significant decrease in the activities of AST, ALT ($p < 0.01$), ALP ($p < 0.001$) and LDH ($p < 0.001$) were observed in the liver of DEN+PB animals compared to control, whereas the activities were increased significantly (AST ($p < 0.01$); ALT, ALP ($p < 0.001$); LDH ($p < 0.001$)) in DEN+PB+ST

Table 1: Levels of CEA and AFP in serum of control and experimental animals carcinoembryonic antigen (CEA) and alpha fetoprotein (AFP) (Values are mean±SD for 6 animals in each group)

Parameters	Months	Control	Drug control (ST)	Induced (DEN+PB)	Induced and treated (DEN+PB+ST)
CEA (ng mL ⁻¹)	3	n.d	n.d	5.12±0.49	2.5±0.25 ^C
	6	n.d	n.d	6.14±0.60	3.01±0.29 ^C
AFP (ng mL ⁻¹)	3	n.d	n.d	167.60±16.5	70.62±6.0 ^C
	6	n.d	n.d	197.51±19.0	59.23±5.80 ^C

nd: Not detected; ^Cp<0.001; Statistically significant variations are compared using Student's t-test Control vs. ST; DEN+PB; DEN+PB vs. DEN+PB+ST diethyl nitrosamine (DEN), Phenobarbital (PB), *Solanum trilobatum* (ST)

Table 2: Activity of marker enzymes - ALT, AST, ALP, LDH and GGT in serum of control and experimental animals (Values are Mean±SD for 6 animals in each group)

Parameters	Months	Control	Drug control (ST)	Induced (DEN+PB)	Induced and treated (DEN+PB+ST)
AST (IU L ⁻¹)	3	24.70±2.50	23.30±2.30 ^{NS}	63.70±6.13 ^C	33.10±3.27 ^C
	6	30.19±2.95	29.30±2.80 ^{NS}	73.73±7.53 ^C	31.10±3.17 ^C
ALT (IU L ⁻¹)	3	14.68±1.33	15.47±1.50 ^{NS}	26.53±2.21 ^C	17.43±1.40 ^C
	6	14.12±1.40	15.18±1.50 ^{NS}	28.90±2.12 ^C	17.45±1.61 ^C
ALP (IU L ⁻¹)	3	19.60±1.90	20.10±1.90 ^{NS}	29.30±2.80 ^C	19.80±1.90 ^C
	6	21.90±2.10	20.30±2.10 ^{NS}	42.60±4.10 ^C	20.00±1.90 ^C
LDH (IU L ⁻¹)	3	30.10±3.1	30.30±3.01 ^{NS}	45.51±4.30 ^C	32.25±3.20 ^C
	6	32.60±3.20	30.70±3.0 ^{NS}	72.30±7.00 ^C	35.40±3.50 ^C
GGT (IU L ⁻¹)	3	18.10±1.70	19.20±1.80 ^{NS}	59.40±5.81 ^C	21.50±2.10 ^C
	6	21.50±1.90	22.30±2.10 ^{NS}	72.50±7.20 ^C	29.70±3.00 ^C

^A-p<0.05; ^B-p<0.01; ^C-p<0.001; NS: Non significant; Statistically significant variations are compared using Student's t-test Control vs. ST; DEN+PB; DEN+PB vs. DEN+PB+ST

Table 3: Activities of marker enzymes - ALT, AST, ALP, LDH, GGT and 5' Nucleotidase in liver of experimental animals (Values are expressed as Mean±SD for 6 animals in each group)

Parameters	Months	Control	Drug control (ST)	Induced (DEN+PB)	Induced and treated (DEN+PB+ST)
AST	3	4.83±0.45	4.80±0.45 ^{NS}	3.12±0.30 ^C	4.52±0.40 ^C
	6	5.92±0.51	5.80±0.55 ^{NS}	3.35±0.30 ^C	4.79±0.50 ^C
ALT	3	6.20±0.60	6.13±0.62 ^{NS}	4.12±0.40 ^C	5.89±0.60 ^C
	6	6.27±0.60	6.25±0.60 ^{NS}	4.27±0.40 ^C	6.07±0.57 ^C
ALP	3	0.75±0.07	0.78±0.08 ^{NS}	0.32±0.02 ^C	0.52±0.05 ^C
	6	0.95±0.08	0.92±0.09 ^{NS}	0.45±0.05 ^C	0.75±0.07 ^C
LDH	3	3.84±0.40	3.5±0.30 ^{NS}	2.07±0.19 ^C	3.17±0.40 ^C
	6	3.91±0.40	3.93±0.4 ^{NS}	2.39±0.50 ^C	3.17±0.30 ^C
GGT	3	3.82±0.40	3.64±0.35 ^{NS}	2.01±0.47 ^C	4.11±0.40 ^C
	6	4.20±0.40	3.92±0.40 ^{NS}	2.20±0.55 ^C	4.23±0.40 ^C
5' Nucleotidase	3	12.10±1.10	11.01±1.01 ^{NS}	22.31±2.17 ^C	15.8±1.40 ^C
	6	15.21±1.37	14.17±1.21 ^{NS}	29.43±2.70 ^C	18.21±1.73 ^C

^A-p<0.05; ^B-p<0.01; ^C-p<0.001; NS: Non significant; Statistically significant variations are compared using Student's t-test Control vs. ST; DEN+PB; DEN+PB Vs DEN+P Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH) γ-Glutamyl transpeptidase (GGT) and Alkaline phosphatase (ALP)

animals compared to DEN+PB animals. Activities of ALP and 5' NT were significantly increased (p<0.001) in DEN+PB animals compared to control and the activities were decreased significantly (p<0.001) in DEN+PB+ST animals compared DEN+PB animals. In ST animals no significant changes in the activity of marker enzymes were observed in liver indicating the non-toxic (Table 3).

DISCUSSION

The anticarcinogenic activity and tumor growth inhibitory effect of sobatum from *Solanum trilobatum* formed the basis for the assessment on the role of *Solanum trilobatum* on DEN induced hepatocarcinogenesis. Previous studies have shown the inhibitory effect of ST on hepatocarcinogenesis (ShahJahan *et al.*, 2007). Thus in addition to its cytotoxicity profile, a biochemical assessment is essential to validate its use as a therapeutic antitumor agent. The present biochemical study clearly depicts the anticancer effect of ST.

AFP and CEA molecule carry the onco-fetal specificity Tumor marker CEA is a glycoprotein that is secreted into the serum in high levels in adenocarcinomas of digestive epithelia. Levels of CEA increase, with an increase in the size of tumor. Serum values of more than 5 ng mL⁻¹ indicate metastasis. Post therapeutic elevations of CEA carry poor prognosis, while a subsequent rise in CEA is suggestive of metastasis.

α - fetoprotein (AFP), is a serum protein that is detected in elevated concentration in conditions like hepatocellular carcinoma. AFP is a serum protein similar in size, structure and amino acid composition to serum albumin, but it is detectable only in minute amounts in the serum of normal adults. Elevated serum concentrations of this protein can be achieved in the adult by exposure to hepatotoxic agents (or) hepatocarcinogens and are frequently associated with HCC. It is a 72 KDa α -1 globulin with an uncertain biological function, is synthesized during embryonic life by foetal yolk sac, liver and intestinal tract. AFP has high specificity for hepatocarcinoma. Its serum concentration can be used to confirm hepatocarcinoma and for the diagnosis of tumor response to therapy. More than 90% of patients with hepatic cancer have increased serum AFP levels.

In the present study, a decrease in the levels of CEA and AFP following ST treatment indicates a positive prognosis (Yoshiji *et al.*, 1991). The decrease in the levels on ST co-treatment prevents the neoplastic growth and reduces hepatic disorder, indicating that it possesses anticarcinogenic properties.

Tumor marker enzymes are indicators of tumor response to therapy. Aminotransferases, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transpeptidase serve as markers of liver damage. Analysis of these marker enzymes in serum and liver reflects the mechanism of cellular damage and subsequent release of proteins, their extracellular turnover and mechanisms of neoplastic processes.

Biochemical tumor markers are used to screen particularly tumor conditions for differential diagnosis, prognosis, monitoring the progress and for assessing the response to therapy (McIntyre and Rosalki, 1992). These enzymes are more unique and changes in their activities reflect the effect of proliferation of cells with growth potential and its metabolic turnover is dramatically different from those of normal cells. The rise in their activities is shown to be in good correlation with the number of transformed cells in cancer conditions.

The role of transamination in biological systems is well known. It is apparent from transaminase substrate, i.e. α -ketoglutaric, oxaloacetic and pyruvic acids on one hand and glutamic and aspartic acids on the other hand, that transamination is concerned with the interconversion of highly important metabolites. Elevated aminotransferase activities levels were observed in cancer bearing animals. Clinical diagnosis of neoplastic patients show an eight times increase over normal control patients (McIntyre and Rosalki, 1992). Transaminase becomes gradually more pronounced towards the terminus, which indicates the severity of an advanced cancer condition. Increased transaminase activities in HCC have been reported by Rocchi *et al.* (1997). A good correlation exists between the activities of ALT and AST of tumor masses during therapy. The stable clinical and enzymatic

pattern of these enzymes is noticed in patients with hepatic malignancy after chemotherapy, while patients failing to respond to drug showed progressive increase in the level of these enzymes. Similar results were observed by in dimethyl hydrazine-induced colon cancer in rats.

Diethylnitrosamine (DEN) is a hepatotoxin and a carcinogen. Similarly PB is hepatotoxin and acts as promotor in hepatocarcinogenesis. An increase in AST, ALT activities in DEN induced animals correlate to the hepatotoxicity and carcinogenesis with the development of preneoplastic changes, increased severity and advanced stage of liver carcinoma. An increase in the serum LDH in malignant liver diseases depends upon the extent of metastasis. The lowering in the activities of AST, ALT and LDH on ST treatment shows the hepatoprotective effect of ST and inhibition of carcinogenesis.

Alkaline phosphatase (ALP) is involved in transport of metabolites across cell membrane, protein synthesis, secretory activities and glycogen metabolism. Alkaline phosphatase is a membrane bound enzyme and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. ALP was noticed in the serum and liver of hepatoma - bearing animals (Patel *et al.*, 1994). Observed that the ALP activity was raised in the serum of cervical carcinoma patients. The rise in a activity of ALP in cancer bearing animals may be due to the disturbance in the secretory activity or in transport of metabolites or may be due to altered synthesis of certain enzymes in these conditions. ALP is used as a specific tumor marker during diagnosis in the early detection of cancer (Kobayashi and Kawakubo, 1994). An increase in ALP activity on DEN administration may be due to altered synthesis of enzymes as in other hepatotoxic condition (Sharma *et al.*, 1995). Activities of ALP are increased in precancerous lesion. In primary liver cell carcinoma and carcinoma of bile duct. The lowering of the activity of these enzymes indicates the inhibition of pre- cancerous transformation in the liver on ST treatment in DEN+PB+ST animals.

Plasma Gamma Glutamyl Transpeptidase (GGT) activity is higher in hepatocellular carcinoma. An increase in GGT activity paralleled with an increase in alkaline phosphatase activity is frequent in hepatocellular carcinoma. (Induction of GGT during hepatocarcinogenesis is frequent. An increased GGT activity causes resorption of GSH by preneoplastic foci rich in GGT that enhances cell proliferation and increases tumor promotion and favors transformation of preneoplastic foci to neoplasia (Stark *et al.*, 1993). Maintenance of GGT in serum on treatment with ST signifies its activities to modulate GSH transport and metabolism and thus prevent hepatocarcinogenesis.

The increase in liver marker enzymes with a corresponding decrease shows the inhibitory effect of *Solanum trilobatum* in DEN induced hepatocarcinogenesis as evident from significant decreases in tumor incidence and tumor markers. The non-toxic effect was also evident from lowering of marker enzymes in serum of ST alone treated animals. The absence of conspicuous side effects suggests that plant extract is safe pharmacologically. Reports are available on the potential of ST as an adjuvant in cancer chemotherapy (Mohan and Devi, 1998a). Hence, the plant extract may prove fruitful for clinical application. However, further studies on its effect on immune status and biochemical pathways are warranted.

5'-Nucleotidase has been reported to be altered in the sera of patients with solid tumors. In human lymphoid system, 5'-nucleotidase is anchored to the plasma membrane and has been described as an important marker for differentiation of B-lymphocytes. In our study, 5'-nucleotidase has been increased significantly in the serum, hepatoma, hepatoma bearing rats. Have demonstrated elevated activity of 5'-nucleotidase in carcinoma of liver, gastrointestinal tract and pancreas. Rosi *et al.* (1998) also observed an increased activity of 5'-nucleotidase in leukemia

patients. During ST treatment the enzyme activity got decreased significantly on time dependent manner.

Lactate dehydrogenase (LDH), a cytoplasmic enzyme which catalyzes the oxidation of lactate to pyruvate and vice versa. LDH, a marker for membrane integrity and is a regulator of many biochemical reactions in the body tissues and fluids have reported that an increased activity of LDH in malignant cells spreading through the organs of tumor bearing rats. The elevated enzyme activity in the serum of patients with lung and ovarian cancer (Bose and Mukherjee, 1994). Depict the changes in permeability of cell membranes and the leakage to soluble enzymes. The possible reason for the elevated activity of LDH in cancer bearing rats may be due to enhanced glycolysis using the growth of tumor. The reduction in LDH activity on treatment with ST controlled the glycolysis and renders protection to membrane integrity.

REFERENCES

- Bailey, N.T.J., 1959. Statistical Method in Biology. The English Univeristy Press, London, pp: 200.
- Bergmeyer, H.U., 1974. Methods of Enzymatic Analysis. Vol. 4, Academic Press, New York, London.
- Bose, C.H. and M. Mukherjee, 1994. Enzymatic tumor markers in ovarian cancer: A multiparametric study. Cancer Lett., 77: 39-43.
- King, J., 1965a. The Transferase-Alanine and Aspartate Transaminase: Practical Clinical Enzymology. Van D. Nostrand Co. Ltd., London, pp: 121-138.
- King, J., 1965b. The Dehydrogenase (or) Oxido-Reductase Lactate dehydrogenase: Practical Clinical Enzymology. Van D. Nostrand Co. Ltd., London, pp: 83-93.
- King, J., 1965c. The Phosphohydrolases-Acid and Alkaline Phosphatases: Practical Clinical Enzymology. Van D. Nostrand Co. Ltd., London, pp: 191-208.
- Kobayashi, T. and T. Kawakubo, 1994. Prospective investigation of tumour markers and risk assessment in early cancer screening. Cancer, 73: 1946-1953.
- Leopky, R.N., 1994. Nitroso Compounds Chemistry and Biochemistry. In: Nitrosamine and Related Compounds: Chemistry and Biochemistry, Leopky, R.N. (Ed.). ACS, Washington DC, pp: 1-19.
- Luly, P., O. Barnabei and E. Tria, 1992. Hormonal control *in vitro* of plasma membrane bound Na^+/K^+ ATPase of rat liver. Biochem. Biophys. Acta., 282: 447-452.
- Macnab, G.M., J.M. Urbanowicz and M.C. Kew, 1978. Carcinoembryonic antigen in hepatocellular cancer. Br. J. Cancer, 38: 51-54.
- Madhavan, S. and S. Balu, 1999. Ethnobotanical studies on *Solanum trilobatum* Linn.: An Indian drug plant. J. Econ. Taxon. Bot., 23: 43-46.
- McIntyre, N. and S. Rosalki, 1992. Biochemical Investigation in the Management of Liver Disease. In: Hepatobiliary Diseases, Prieto, J., J. Rodes and D.A. Shafritz (Eds.). Springer-Verlag, Berlin, pp: 39-71.
- Mohan, P.V. and K.S. Devi, 1996. Cytotoxic potential of the preparations from *Solanum trilobatum* and the effect of sobatum on tumor reduction in mice. Cancer Lett., 110: 71-76.
- Mohan, P.V., K. Rathinam and K.S. Devi, 1996. Lack of micronucleus induction by sobatum in bone marrow erythrocytes of Swiss mice. Mut. Res., 361: 23-27.
- Mohan, P.V. and K.S. Devi, 1997a. Effect of sobatum on tumor development and chemically induced carcinogenesis. Cancer Lett., 112: 219-223.

- Mohanani, P.V. and K.S. Devi, 1997b. Mutagenicity and antimutagenicity evaluation of sobatum by *in vitro* method. Biomedicine, 17: 9-15.
- Mohanani, P.V. and K.S. Devi, 1998a. Chemoprotective effect of Sobatum against cyclophosphamide toxicity in mice. J. Exp. Clin. Cancer Res., 17: 159-164.
- Mohanani, P.V. and K.S. Devi, 1998b. Effect of Sobatum on radiation-induced toxicity in mice. Cancer Lett., 123: 141-145.
- Patel, P.S., G.N. Rawal and D.B. Bala, 1994. Combined use of serum enzyme levels as tumour markers in cervical carcinoma patients. Tumor Biol., 15: 45-51.
- Rocchi, E., Y. Seium, L. Camellini, G. Casagrandi, A. Borghi, P. D'Alimonte and G. Cioni, 1997. Hepatic tocopherol content in primary hepatocellular carcinoma and liver metastases. Hepatology, 26: 67-72.
- Rosi, F., A. Tabucchi, F. Carlucci, P. Galieni and F. Laukia *et al.*, 1998. 5-nucleotidase activity in lymphocytes from patients affected by B-cell chronic lymphocytic leukemia. Clin. Biochem., 31: 269-272.
- Shahjahan, M., K.E. Sabitha, M. Jainu and C.S. Shyamala Devi, 2004. Effect of Solanum trilobatum against carbon tetrachloride induced hepatic damage in albino rats. Ind. J. Med. Res., 120: 194-198.
- Shahjahan, M., G. Vani and C.S. Shyamaladevi, 2005. Effect of Solanum trilobatum on the antioxidant status during diethyl nitrosamine induced and phenobarbital promoted hepatocarcinogenesis in rat. Chem. Biol. Interact., 156: 113-123.
- Shahjahan, M., G. Vani and C.S. Shyamaladevi, 2007. Effect of Solanum trilobatum on hepatic drug metabolising enzymes during diethylnitrosamine-induced hepatocarcinogenesis promoted by Phenobarbital in rat. Hepatol. Res., 37: 35-49
- Sharma, A., R. Mathur and S. Skukla, 1995. Hepatoprotective action of a proprietary herbal preparation against carbon tetrachloride intoxication. Indian Drugs, 32: 120-124.
- Stark, A.A., E. Zeiger and D.A. Pagano, 1993. Glutathione metabolism of gamma glutamyl transpeptidase leads to lipid peroxidation, characterization of the system and relevance to hepatocarcinogenesis. Carcinogenesis, 14: 183-189.
- Yoshiji, H., D. Nakae, T. Kinugasa, M. Matsuzaki, A. Denda, T. Tsujii and Y. Konishi, 1991. Inhibitory effect of dietary iron deficiency on the induction of putative preneoplastic foci initiated with diethylnitrosamine and promoted by phenobarbital. Br. J. Cancer, 64: 839-842.