ORIGINAL ARTICLE

Hepatoprotective activity of *Amomum subulatum* Roxb against ethanol-induced liver damage

Mihir Y. Parmar, Purvi Shah, Vaishali Thakkar, Tejal R. Gandhi
Anand Pharmacy College, Shree Ramkrishna Seva Mandal Campus, Anand-388001, Gujarat, India

The hepatoprotective activity of methanolic extract of *Amomum subulatum* Roxb (Zingiberaceae) seeds was studied against 20 % ethanol (3.76 g/kg/days, p.o for 18 days) induced liver damage in rats. Ethanol produced significant changes in various liver parameters such as functional (thiopentone-induced sleeping time) and physical (increased liver weight and volume). It also increased the biochemical parameters such as serum glutamate oxaloacetic transaminase and glutamate pyruvic transaminase, alkaline phosphatase, total and direct bilirubin, total cholesterol, triglyceride and decreased total protein along with changes in histological parameters (damage to hepatocytes). Treatment with methanolic extract of *A. subulatum* (100 and 300 mg/kg/day, p.o. for 18 days) and silymarin significantly prevented the functional, physical, biochemical and histological changes induced by ethanol, indicating the recovery of hepatic cells. These results demonstrate that methanolic extract of *A. subulatum* seeds possessed the hepatoprotective activity.

Key words: *Amomum subulatum*, hepatoprotective activity, ethanol

INTRODUCTION

Alcohol toxicity is one of the world's major health problems, as the significant numbers of peoples are affected due to several fatal diseases caused by alcohol. Herbal alternatives are one of the best ways to minimize these disease conditions. For this we have chosen ethanol-induced toxicity in rat model to study effect of ethanol on liver.[1]

As an herbal alternative, we have used *Amomum subulatum* Roxb. It also known as greater cardamom of Zingiberaceae family. It is a perennial herb, which grows widely in moist tropical countries. Its seeds are used as flavouring spices, cardiac tonic, expectorant and diuretic. It is also used in anorexia, dyspepsia, hyperacidity, dysentery, skin diseases, wounds, ulcers, cardiac debility, liver congestion, cough, fever and gonorrhoea.[2] It is one of the plants mentioned in the literature having claims of activity against liver disorders.[3] In the light of above fact, the present study was carried out to evaluate the hepatoprotective activity of methanolic extract of *A. subulatum* seeds.

MATERIALS AND METHODS

Plant Material and Extraction

A. subulatum fruits were purchased from a local market of Anand, India, during July 2007. The fruits were identified and authenticated by Dr. A. S. Reddy, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India where a voucher specimen (No. MP-2: 28/7/2007) was kept for future reference. The seeds were dried at room temperature and mechanically powdered to obtain a coarse powder; defatted with petroleum ether (60–80°C) and Soxhlet extracted with methanol. Solvent removal under reduced pressure was afforded by methanolic extract (yield 9.5% w/w). The dry methanolic extract was stored in cool and dry place which was further used for evaluation of the hepatoprotective activity.

Phytochemical Screening

A preliminary phytochemical screening of methanolic extract of *A. subulatum* seeds was carried out.[4]

Animals

Studies were carried out using either sex Wistar albino rats (200–220 g). They were obtained from the animal house, Anand pharmacy college (APC), Anand, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions; temperature (22 ± 2°C), relative humidity (55 ± 5 %) with dark and light cycle (12/12 hours). They were allowed free access of standard pellet diet (Amrut feed, Sangli, India) and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before the commencement of experiment. Animal studies were approved by the Institutional Animal Ethics

Address for correspondence: Dr. Mihir Y. Parmar, Anand Pharmacy College, Shree Ramkrishna Seva Mandal Campus, Opp. Town Hall, Anand-388001, Gujarat, India. E-mail: mihirdarji4u@yahoo.co.in
Received: 03-07-2008; **Accepted:** 05-10-2008; **DOI:** 10.4103/0973-8258.56286

Committee (Protocol No. 7004 dated 7August 2007) and conducted according to the regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and Chemicals

Ethanol was purchased from Baroda Chemical Industries Ltd, Dabhoi. Silymarin was obtained as a gift sample from Micro labs, Bangalore, India. SGOT, SGPT, ALP, Bilirubin and TC kits were procured from Span Diagnostics, Surat, India. TG kits were procured from Reckon diagnostic Pvt. Ltd, Baroda, India. All other chemicals and reagents used were of analytical grade.

Acute Toxicity Studies

Acute toxicity study was conducted for methanolic extract of *A. subulatum* seeds by the stair case method following OECD guidelines-425, 2001.[5] There was no lethality observed up to a dose of 3000 mg/kg. One-tenth of maximum, i.e., 300 as well as 100 mg/kg doses were selected for the evaluation.[6]

Experimental Protocols

Animals were randomly divided into five groups with six each. All animals except the normal control group were intoxicated with 20% ethanol (3.76 g/kg/day, p.o for 18 days). Group I (normal control) received only distilled water and Group II (ethanol control) received 20% ethanol (3.76 g/kg/day, p.o. for 18 days). Group III (Test-1) and Group IV (Test-2) received methanolic extract of *A. subulatum* at 100 mg/kg and 300 mg/kg, p.o., respectively, while Group V (positive control) received silymarin (200 mg/kg, p.o.).[7] On day 19, thiopentone sodium (40 mg/kg, i.p) was injected and the sleep time was recorded in all the animals.

After complete recovery from thiopentone sodium effect, blood was collected from retro-orbital plexus of overnight fasted rats. The blood samples were allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at 2500 rpm at 4°C for 15 minutes and used for the estimation of various biochemical parameters such as SGOT and SGPT,[8] ALP,[9] TBL and DBL,[10] TC,[11] TG,[12] and TP.[13]

After collection of blood samples, the rats were sacrificed by ether anaesthesia and their livers were excised, rinsed in ice-cold normal saline and the wet-weight and volume were determined. Histology of liver was carried out.[14] In brief, one animal from the treated groups showing maximal activity as indicated by improved functional, physical and biochemical parameters from each test, positive control and ethanol control, were utilized for these purposes. The animals were sacrificed, and the abdomen was cut open to remove the liver. Sections of 5 μm liver were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 hours, then embedded in paraffin and stained using haematoxylin–eosin (H and E) dye, finally mounted in diphenylxylene. The sections were observed under the microscope for histological changes in liver architecture and their photomicrographs were taken.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's procedure.[15] *P* values <0.05 were considered to be significant.

RESULTS

Preliminary phytochemical screening of the methanolic extract of *A. subulatum* seeds revealed the presence of flavonoids, terpenoids, glycosides and volatile oils. The methanolic extract of *A. subulatum* seeds was found to be nontoxic up to a dose of 3000 mg/kg.

A significant increase in the functional parameter such as thiopentone-induced sleep time and physical parameters such as weight and volumes of wet liver were found in ethanol control as compared to normal control. Treatments with Test-1, Test-2 and positive control caused a significant decrease in functional and physical parameters as compared to ethanol control [Table 1]. A significant increase in biochemical parameters such as SGOT, SGPT, ALP, TBL, DBL, TC, TG and decreased TP levels were found in ethanol control as compared to normal control. Treatments with Test-1, Test-2 and positive control caused a significant reversal in above biochemical parameters as compared to ethanol control [Table 2].

Table 1: Effect of the methanolic extract of *A. subulatum* seeds on physical and functional parameters

Groups	Thiopentone induced sleeping		Mean liver weight (g/100 g)	Mean liver volume (ml/100 g)
	Onset (seconds)	Duration (minutes)		
I–Normal control	172.7 ± 3.55	41.7 ± 1.05	3.84 ± 0.30	4.42 ± 0.33
II–Ethanol control	118.3 ± 4.59##	144.0 ± 6.56##	5.44 ± 0.14##	5.35 ± 0.18##
III–Test-1	165.2 ± 3.72*	90.3 ± 3.06*	3.83 ± 0.14*	3.76 ± 0.12*
IV–Test-2	170.5 ± 1.77*	81.8 ± 3.91*	3.76 ± 0.18*	3.64 ± 0.13*
V–Positive control	161.0 ± 1.77*	58.3 ± 2.33	3.80 ± 0.15*	3.58 ± 0.24*

Values are expressed as mean ± SEM; n = 6 rats in each group; #*P* <0.05 and ##*P* <0.001 are considered significant when compared with Group I; **P* <0.05 is considered significant when compared with Group II by Dunnett's multiple comparison test.

Table 2: Effect of methanolic extract of *A. Subulatum* seeds on biochemical parameters

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (KAU/dl)	TBL (mg/dl)	DBL (mg/dl)	TC (mg/dl)	TG (mg/dl)	TP (mg/ml)
I - Normal control	33.5 ± 4.7	28.2 ± 2.2	7.2 ± 0.7	0.36 ± 0.04	0.14 ± 0.02	89.5 ± 4.7	65.6 ± 8.8	7.69 ± 0.8
II - Ethanol control	169.7 ±12.4$^{\#\#}$	125.3 ± 6.8$^{\#\#}$	99.7 ± 6.8$^{\#\#}$	1.53 ± 0.14$^{\#\#}$	0.84 ± 0.04$^{\# \#}$	170.1 ± 15.3$^{\#\#}$	217.5 ± 3.4$^{\#\#}$	2.99 ± 0.6 $^{\# \#}$
III - Test-1	101.7 ± 8.9*	66.3 ± 3.9*	32.7 ± 11.6*	0.61 ± 0.04*	0.39 ± 0.04*	90.9 ± 2.7*	120.79 ± 3.9*	5.30 ± 0.3*
IV - Test-2	89.2 ± 5.3*	61.0 ± 3.4*	27.1 ± 3.5*	0.54 ± 0.08*	0.30 ± 0.03*	84.3 ± 11.7*	96.9 ± 6.9*	5.97 ± 0.2*
V - Positive control	78.0 ± 7.7*	47.0 ± 5.2*	20.5 ± 1.9*	0.42 ± 0.06*	0.29 ± 0.03*	94.3 ± 4.14*	88.5 ± 8.6*	6.07 ± 0.1*

Values are expressed as mean ± SEM; n=6 rats in each group; # P<0.05 and # # P<0.001 are considered significant when compared with group I; * P<0.05 is considered significant when compared with group II by Dunnett's multiple comparison test.

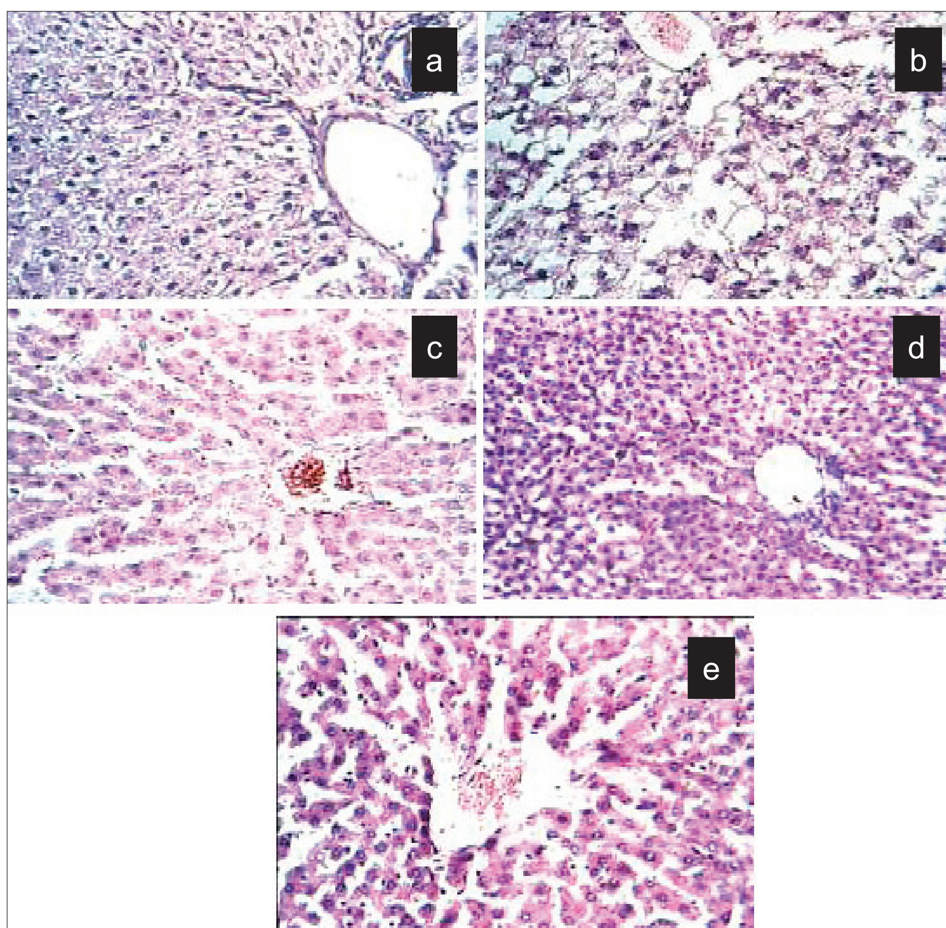


Figure 1: Photomicrograph of rat liver obtained from different treatment groups. (a) Control; (b) ethanol control; (c) Test-1; (d) Test-2; (e) positive control (H and E, ×100)

The histology of the normal control showed a normal architecture of the liver [Figure 1a]. In ethanol control, there was a microvascular fatty change and the hepatocytes were surrounded by large number of fat droplets [Figure 1b]. Treatment with Test-1, Test-2 and silymarin showed minimal fatty changes [Figure 1c–e] and their architectures were normal, indicating the hepatoprotective effect of these extracts. However, Test-2 showed less microvascular fatty changes than Test-1.

DISCUSSION

The liver can be injured by many chemicals and drugs. Ethanol was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. Ethanol produces dose-related deleterious effects in the liver.[16]

Ethanol alters the metabolic activity of hepatocytes, thereby inducing hepatic damage. Barbiturates are a class of xenobiotics that are extensively metabolized in the liver. Deranged liver function leads to delay for the clearance of barbiturates, resulting in a longer duration of a hypnotic effect.[17] In the present study, administration of thiopentone sodium to rats pretreated chronically with alcohol resulted in an increased duration of thiopentone sodium sleep time. Treatments with the methanolic extract of *A. subulatum*

seeds significantly decreased thiopentone-induced sleep time, an indirect evidence of their hepatoprotective activity.

In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes,[18] with an impaired protein secretion by hepatocytes.[19] Water is retained in the cytoplasm of hepatocytes leading to the enlargement of liver cells, resulting in increased total liver mass and volume.[20] Treatments with the methanolic extract of *A. subulatum* seeds significantly reduced the total wet liver weight and volume, thus indicating their hepatoprotective activity.

During hepatic damage, cellular enzymes like SGOT, SGPT and ALP present in the liver cells leak into the serum, resulting in increased concentrations.[21] Ethanol administration for 18 days significantly increased all these serum enzymes, whereas treatments with the methanolic extract of *A. subulatum* seeds significantly reduced SGOT, SGPT, ALP and increased TP levels indicating their hepatoprotective activity.

Ethanol also induces hypercholesteraemia and hypertriglyceridaemia, which may be due to the activation of enzyme HMG Co-A reductase, the rate-limiting step in cholesterol biosynthesis. The increased serum triglyceride level in ethanol control may be due to the decreased activity of lipoprotein lipase, which is involved in the uptake of triglyceride-rich lipoprotein by the extrahepatic tissues.[22] Treatments with the methanolic extract of *A. subulatum* seeds significantly reduced the TC and TG levels, suggesting that the extracts prevented ethanol-induced hyperlipidaemia probably due to their hepatoprotective activity.

Histological changes such as steatosis and perivenular fibrosis were observed in ethanol control. Treatments with the methanolic extract of *A. subulatum* seeds prevented these histological changes, further indicating their hepatoprotective activity.

Ethanol, even after short-term consumption, induces CYP2E1 enzyme activity in doses that do not cause fatty changes. This enzyme accelerates alcohol metabolism with a resultant increase in acetaldehyde production.[23] Acetaldehyde is thought to have a number of adverse effects like decreased transport and secretion of proteins due to tubulin polymerization, enhanced vitamin metabolism, and trace metals and drugs like paracetamol cause severe acute liver injury which is sometimes fatal.[24–26] Antioxidants exhibit hepatoprotective activity by blocking the conversion of ethanol to acetaldehyde.[27]

Phytoconstituents like flavonoids, terpenoids, glycosides and volatile oils[28–31] are well known for their antioxidant and hepatoprotective activities. Preliminary phytochemical screening of the methanolic extract of *A. subulatum* seeds contain above-mentioned phytoconstituents, which may be attributed to the individual or combined effect of phytoconstituents present in it. Further, investigation is underway to determine the exact phytoconstituent that is responsible for activities.

It can be concluded that the methanolic extract of *A. subulatum* seeds possessed the hepatoprotective activity against ethanol-induced liver damage in rats, as evidenced by the functional, physical, biochemical and histological parameters.

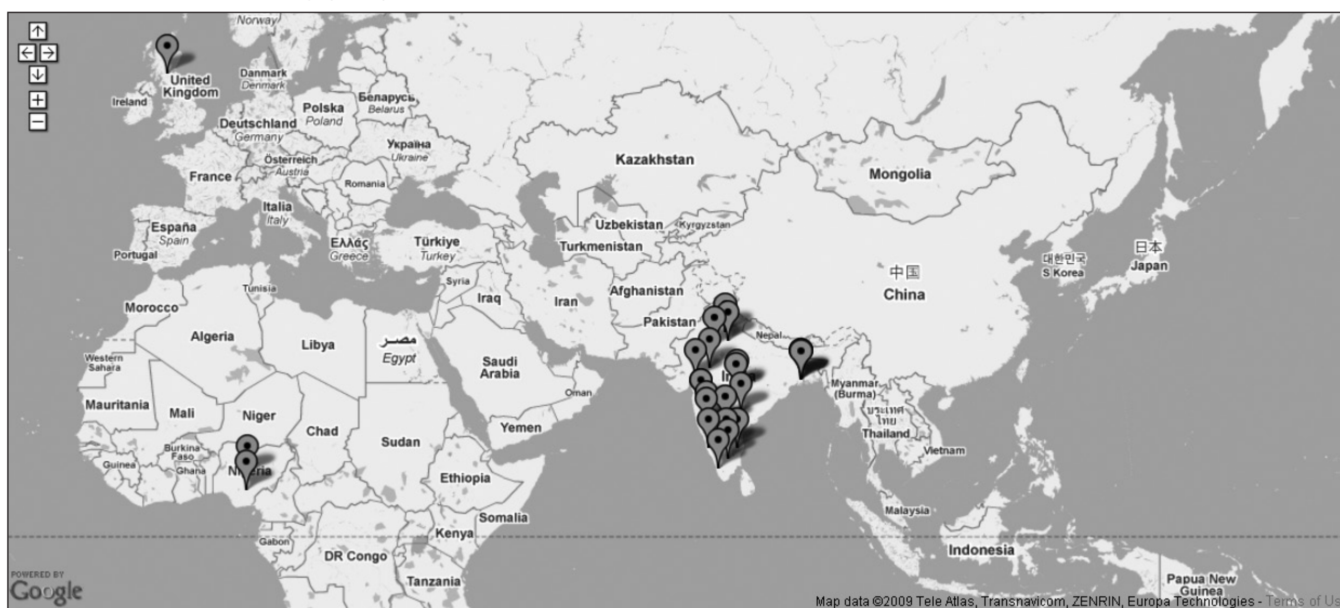
REFERENCES

1. Singha PK, Roy S, Dey S. Protective activity of andrographolide and arabinogalactan proteins from Andrographis paniculata Nees against ethanol-induced toxicity in mice. J Ethnopharmacol 2007;111:13–21.
2. Sharma PC, Yelne MB, Dennis TJ, Database on medicinal plants used in ayurveda. 1st ed, Vol. 2. New Delhi: CCRAS; 2002. p. 454-61.
3. Warrier PK, Nambiar VP, Ramankutty C. Indian Medicinal Plants a compendium of 500 species, 1st ed, India: Orient Longman Pvt Ltd; 1994. p. 128-31.
4. Khandelwal KR, Practical Pharmacognosy Techniques and Experiments, 16th ed, Pune: Nirali Prakashan; 2006. p. 149-56.
5. OECD 2001-guideline on acute oral toxicity (AOT), Environmental health and safety monograph series on testing and adjustment, No. 425.
6. Handa SS, Sharma A. Heptoprotective activity of andrographolide from Andrographis paniculata against Ethanol. Indian J Med Res 1990;92:276.
7. Shukla V, Tiwari RK, Agarwal DP. Hepatoprotective role of Picroliv isolated from Picrohiza kurroa on alcohol induced liver necrosis. Adv Pharmacol Toxicol 2001;2:9-16.
8. Reitman S, Frankel S. A Colorimetric method for the determination of serum glutamate oxaloacetic transaminase and glutamate pyruvic transaminase. Am J Clin Pathol 1957;28:56-63.
9. Kind PR, King EJ. Estimation of plasma phosphatases by determination of hydrolyzed phenol with antipyrine. J Clin Pathol 1954;7:322-30.
10. Malloy HJ, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937;119:481.
11. Kaplan A, Lavernel LS. Lipid metabolism. In Clinical chemistry: Interpretation and techniques, 2nd ed, Philadelphia: Lea Febiger; 1983. p. 333-6.
12. Fossati P, Lorenzo P. Serum triglycerides determined colorimeterically with an enzyme that produces hydrogen peroxide, Clin Chem 1982;28:2077-80.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1957;193:265-75.
14. Galighor AE, Koyloff EN, Essentials of practical micro technique, 2 ed, Philadelphia: Lea and Febiger; 1976. p. 210.
15. Sanford B, Gennaro AR, Eds. Remington; The science and practice of pharmacy, 19th ed. Easton, PA: Mack Publishing Company; 1995. p. 111.
16. Leo MA, Arai M, Hepatotoxicity of vitamin A and ethanol in rat. Gastroenterology 1982;82:194-205.

17. Kulkarni SK. Handbook of experimental pharmacology, 3rd ed, New Delhi: Vallabh Prakashan; 1999. p. 115-6.
18. Kumary AL, Vijayammal PL. Additive effect of alcohol and nicotine on lipid metabolism in rats. Indian J Exp Biol 1993;31:270-4.
19. Vilstrup H, Tygstrup N. Glucoregulation in acute liver failure. Eur J Clin Investig 1985;16:193-7.
20. Blancho CD, Gentil S. Alterations of glucose metabolism in chronic liver disease. Diabetes Res Clin Pract 1990;8:29-36.
21. Deb AC. Fundamentals of biochemistry, 7th ed. Kolkata, India: New Central Book Agency; 2002. p. 139-40.
22. Younes M, Strubelt O, Alcohol induced hepatotoxicity: A role for oxygen free radicals. Free Radiac Res Common 1987;3:19-26.
23. Lieder CS, DeCarli LM, Hepatic Microsomal ethanol oxidzing system: *In vitro* characterisitcs and adapative properties *in vivo*. J Biochem 1970;245: 2505-12.
24. Fromenty B, Pessayre D. Impaired mitochondrial function in microvesicular steatosis: Effect of drugs, ethanol, hormones and cytokines, J Hepatol 1997;26:43-53.
25. McClain CJ, Kromhount JP. Potentiation of acetaminophen hepatotoxicity by alcohol. JAMA 1980;244:251-3.
26. Seeff LB, Adler E. Acetaminophen hepatotoxicity in alcoholics: A therapeutic misadventure. Ann Intern Med 1986;104:399-404.
27. Maneesh M, Jayalakshmi H, Effect of ascorbic acid, alpha tocopherol, lecithin and L-ornithine-L-asparate on ethanol induced hypoproteinemia and hyperlipidaemia in rats. Indian J Physiol Pharmacol 2005;49:422-6.
28. Defeudis FV, Papadopoulos V, Drieu K, Ginkgo biloba extracts and cancer: A research area in its infancy. Fundam Clin Pharmacol 2003;17:405-17.
29. Takeoka GR, Dao LT. Antioxidant constituent of almond [Prunus dulcis (Mill). Webb DA] Hulls. J Agric Food Chem 2003;51:496-501.
30. Quyang MA, He ZD, Wu CL. Antioxidative activity of glycosides from Ligustrum sinense. Nat Prod Res 2003;17:381.
31. Hikino H, Tohkin M, Kiso Y, Namiki T, Nishimura S, Takeyama K, Antihepatoxic actions of Allium sativum bulbs. Planta Med 1986;53:163.

Source of Support: Nil, **Conflict of Interest:** None declared.

Author Institution Mapping (AIM)



Please note that not all the institutions may get mapped due to non-availability of the requisite information in the Google Map. For AIM of other issues, please check the Archives/Back Issues page on the journal's website.