Global Journal of Medical research
Pharma, Drug Discovery, Toxicology and Medicine
Volume 13 Issue 2 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Comparative Hepatoprotective Activities of Selected Indian Medicinal Plants

By Mohammed Fazil Ahmed & A. Srinivasa Rao
Nizam Institute of Pharmacy & Research Center

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Keywords : *melia azedarach linn, catharanthus rosea and brassica oleracea L.var.capitata, hepatoprotective activity and simvastatin.*

GJMR-B Classification : *NLMC Code: WB 55.A9*

Strictly as per the compliance and regulations of:

Comparative Hepatoprotective Activities of Selected Indian Medicinal Plants

Mohammed Fazil Ahmed [α] & A. Srinivasa Rao [σ]

Abstract - The present study deals with comparative evaluation of hepatoprotective activities of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* ethanolic leaves extracts against simvastatin induced hepatotoxicity in rats. Hepatotoxicity in rats was induced by simvastatin (20 mg/kg p.o. for 30 days) and the protective effect of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* (300 mg/kg/p.o. and 500 mg/kg/p.o.) was identified by estimating marker enzymes. Simvastatin treated rats shows significant changes in biochemical parameters i.e. increases in Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alanine Phosphatase (ALP), Serum bilirubin and decrease in Total proteins content, which were restored towards normalization in extracts treated rats. The results revealed that the ethanolic extracts of *Melia azedarach* Linn, and *Brassica oleracea L.var.capitata* (300 mg/kg/p.o. and 500 mg/kg/p.o.) exhibited potent hepatoprotective activity than *Catharanthus Rosea*. The hepatoprotective effect of extracts was further confirmed by histopathological studies of liver, which shows normal architecture of liver cell than compared with hepatotoxicant group. Possible mechanism for hepatoprotective activity may be due to free radical scavenging potential in extracts.

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I. Introduction

Liver diseases remain as one of the serious health problems. It is the key organ of metabolism and excretion. It is often exposed to variety xenobiotics and therapeutic agents.[1] Conventional drugs used in the treatment of liver diseases are often inadequate. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety. More than 900 drugs have been implicated in causing liver injury[2] and it is the most common reason for a drug to be withdrawn from the market. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.[3, 4]

Simvastatin hepatotoxicity is hypothesized to occur due to drug-drug interactions.[5, 6] Simvastatin (Lipid Lowering Agent) competitively inhibits HMG-Co A (3-hydroxy-3 methylglutaryl coenzyme A) to mevalonate. Mevalonate is also a precursor of Coenzyme Q10 (CoQ10). Thus, treatment with statins could also lower its levels. CoQ10 acts as an antioxidant, has membrane stabilising effects, and is important for cellular mitochondrial respiration, which is essential for energy production in organs.[7, 8]. Thus, simvastatin causes oxidative stress mediated hepatotoxicity by depleting antioxidant enzymes.[9] The enzymes L- alanine aminotransferase (L-ALT), L-aspartate amino transferase (L-AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), are often used in assessing the integrity of the liver.[10]Liver fibrosis occurs as a result of a variety of pathological factors, including viral hepatitis (especially hepatitis B and C), alcohol and drug abuse, metabolic diseases due to overload of iron or copper, autoimmunity against hepatocytes or bile duct epithelium and congenital abnormalities.[11]

Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India. Silymarin is a flavonolignan that has been introduced fairly recently as a hepatoprotective agent. Silymarin is extracted from the seeds and fruits of *Silybum marianum* and in reality is a mixture of three structural components: Silibinin, Silydianine and Silychristine. Clinical trials have shown that Silymarin exerts hepatoprotective effects in acute viral hepatitis, poisoning by Amanita *phalloides,* ethanol, paracetamol, and carbon tetrachloride. Many studies have demonstrated the beneficial hepatoprotective effects when treatment with Silymarin.[12]

Catharanthus Rosea, which is commonly known as '*Vinca rosea*' belongs to family Apocynaceae and is an important source of indole alkaloids, which are present in all plant parts. The important antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine, and reserpine are reported to be present in the roots.[13] Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia.[14, 15]

Melia azedarach, the Persian Lilac is popularly known as Maha neem tree and cultivated in all stations. It is a large evergreen tree found throughout India and very similar to Neem. Leaves have been shown to

Author α : Nizam Institute of Pharmacy & Research Center, Deshmukhi, Pochampally (M), Near Ramoji Film City, Nalgonda,(AP), India-508284. E-mail : mohdfazil_pharma@yahoo.co.in
Author σ : Bhaskar Pharmacy College,Yeknapally, Moinabad (Mandal), R.R(Dist), Hyderabad-500075.

contain nimbinene, meliacin, quercetrin, quercetin-3-0-b-rutinoside, kaempferol- 3-0-b rutinoside, rutin and kaempferol-3-L-rhamno-Dglucoside.[16,17] Hot methanolic etract of *Melia azedarach* leaves contain dipentadecyl ketone, glycerol 1,3-bis-undec-9- enoate 2-dodec-9-enoate and glycerol tris-tridec-9-enoate.[18] The plant is traditionally used for the treatment of leprosy, inflammations, and cardiac disorders. Its fruits extracts possess ovicidal[19] and larvicidal activity. [20] The leaf extracts also possess antiviral [21] and antifertility activity.[22]

Brassica oleracea var. capitata (Cabbage) (Family Brassicaceae) is an excellent source of vitamin C. It also contains significant amounts of glutamine, an amino acid that has anti-inflammatory properties. Cabbage can also be included in dieting programs, as it is a low calorie food.The present study was directed to investigate the hepatoprotective activities of *Brassica oleracea L. var. capitata* against simvastatin induced hepatotoxicity**.** There are increasing evidences that increased consumption of fruits and vegetables and intake of certain non-nutrients that are present in foods reduce the risk of various pathological events such as cancer[23,24] and cardio- and cerebro-vascular diseases.[25] The vegetables are rich sources of many nutrients and antioxidant vitamins. Therefore, the objectives of present study deals with comparative evaluation of hepatoprotective activities of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* ethanolic leaves extracts against simvastatin induced hepatotoxicity in rats.

II. Materials and Method

a) Plant collection and identification

The plant material of *Brassica oleracea var. capitata (Cabbage)* ware obtained from local market, while *Melia azedarach Linn* and *Catharanthus Rosea* were obtained from Mount Opera Garden, Near Ramoji Film City, Nalgonda Dist. The plant can be identified authenticated by Department of Botany, research office (Botanist), Anwar-ul- loom College of Pharmacy, Hyderabad.

b) Extraction

The leaves of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* were dried under shade and powdered in a mechanical grinder. The powdered material (250gms) was extracted successively in Ethanol using Soxhlet apparatus at 55°C for 18 h. The extracts was concentrated in vacuo and kept in a vacuum dessicator for complete removal of solvent and weighed.

c) Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of NIZAM INSTITUTE OF PHARMACY, Deshmukhi, Ramoji film city, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiment were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee).

d) Acute toxicity studies of extracts

Acute oral toxicity was performed as per Organiza-tion for Economic Co-operation and Development (OECD)-423 guidelines.[26] Three male Wistar rats weighing between 150–200 g were used for each dose. The dose levels of 5 mg, 50 mg, 500 mg, 1000 mg, 2000 mg, and 5000 mg/kg/body weight, per os were selected. The lethal dose (LD)-50 value of the extract was determined. The drug was administered orally to rats, which were fasted overnight with water ad libitum before the administration of the drug. The body weight of the rat was noted before and after treatment. The animals were observed for toxic symptoms, such behavioral changes, loco-motion, convulsions, and mortality for 72 hours.

e) Experimental design for hepatoprotective activity[27]

Nine groups of rats, six in each received the following treatment schedule.

Group I : Normal control (saline)
Group II : Simvastatin (SMT) (20mg/kg.p.o)
Group III : Simvastatin (20mg/kg.p.o) + *Melia azedarach* leaf extract (300mg/kg, p.o)
Group IV : Simvastatin (20mg/kg.p.o)+*Melia azedarach* leaf extract (500mg/kg, p.o)
Group V : Simvastatin (20mg/kg.p.o) + *Catharanthus roseus* leaf extract (300mg/kg, p.o)
Group VI : Simvastatin (20mg/kg.p.o) + *Catharanthus roseus* leaf extract (500mg/kg, p.o)
Group VII: Simvastatin (20mg/kg.p.o) + *Brassica oleracea L.* var *capitata* leaf extract (300mg/kg, p.o)
Group VIII: Simvastatin (20mg/kg.p.o) + *Brassica oleracea L.* var *capitata* leaf extract (500mg/kg, p.o)
Group IX : Simvastatin (20mg/kg.p.o) +Silymarin (25mg/kg, p.o)

Animals were divided into nine different groups, each having 6 rats and treated accordingly. Group 1: rats fed with a normal standard diet for 30 days; Group II rats receives Simvastatin (SMT) (20mg/kg.p.o alone for 30 days); Group III and IV rats receive SMT along with *Melia azedarach* leaf extracts(300mg/kg and 500mg/kg.p.o respectively for 30days); Group V and VI rats receives SMT along with *Catharanthus roseus* leaf extract (300mg/kg and 500mg/kg p.o respectively for 30days); Group VII and VIII rats received SMT along with *Brassica oleracea* leaf extracts (300mg/kg and

500mg/kg p.o respectively for 30days) and Group IX rats receive SMT along with silymarin (20 mg/kg/p.o.for 30 days).

f) Blood Biochemistry

Blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT [28], ALP [29] and bilirubin [30] by standard method. Serum total protein was measured according to the method of Lowry et al, 1951. [31]

g) Histopathology

Histopathology of liver was carried out by a modified Luna (Luna LG., 1999) [32]. In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 h. Then the livers were paraffin embedded and 5 µ thickness microtone sections were made.[33] The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light micro-scope for any histological damage/protection.

h) Statistical Analysis

The data are represented as mean ± S.E.M. Students't-test is used for statistical analysis of blood serum parameters and for statistical analysis of liver enzymes

III. Results

The acute oral toxicity study of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* showed no mortality upto 2000 mg/kg.The effect of ethanol extracts of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* on serum transaminases, alkaline phosphates, bilirubin and total protein level in Simvastatin intoxicated rats are summarized in Table 1. There was a significant increase in bilirubin levels, SGPT, SGOT and ALP, in Simvastatin intoxicated group(Group II) compared to the normal control group(Group I), while the total protein levels were significantly decreased from the level of 6.46 g/dl to 3.31g/dl in Simvastatin intoxicated rats. Extracts treatment groups such as Group III to Group VIII and standerd group such as Group IX (Simvastatin 20mg/kg.p.o + Silymarin 25mg/kg, p.o) showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 1).

From the above results (Table1), it shows that ethanolic extracts of ***Melia azedarach*** and *Brassica oleracea L. var. Capitata* (300mg/kg, and 500mg/kg,) are more effiective than ***Catharanthus roseus*** (300mg/kg, and 500mg/kg,) of which *Brassica oleracea L. var* (500mg/kg,) is shown to be more effective than ***Melia azedarach*** (500mg/kg,) in restoration of the above parameters to normal level.

Table 1 : Effect of various groups on some serum chemical parameters

Groups	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)	Total bilirubin (mg/dl)	Total protein(g/dl)
Group I	34.32±0.75	36.89±2.30	71.45 ± 0.22	0.40±0.02	6.46±0.02
Group II	126.9±1.50[a, **]	179.95±1.350[a, **]	172.68±0.64[a, **]	1.96±0.12[a,**]	3.31±0.08[a,**]
Group I11	83.2±0.27[b,**]	123.56±0.750[b,**]	133.0±1.63[b,**]	0.68±0.08[b,**]	4.08±0.15[b,**]
Group IV	46.6±0.55[b,**]	68.63±0.82[b, **]	98.0±1.24[b,**]	0.56±0.01[b,**]	6.12±0.15[b,**]
Group V	112.2±0.60[b,*]	168.01±2.75[b,*]	162.2±2.42[b,*]	1.42±0.03[b,*]	3.98±0.06[b,*]
Group VI	98.6±2.38[b,**]	142.58 ±2.53[b,**]	118.24±1.88[b, **]	0.99±0.05[b,**]	4.41±0.02[b,**]
Group VII	48.6±1.62[b,**]	70.23±1.34[b,**]	93.6±0.53**	0.58±0.06[b,**]	5.45±0.08[b,**]
Group VIII	38.23±1.42[b, **]	39.09±1.60[b,**]	74.3±1.72***	0.45±0.04[b,**]	6.24±0.07[b,**]
Group IX	36.98±2.74[b, **]	38.67±1.25[b,**]	72.6±1.04[b,**]	0.42±0.02[b,**]	6.50±0.12[b,**]

Values are mean ± SEM (n=6) one way ANOVA followed by Dunnet's't' multiple Comparison test. a - Group I vs. Group II; b – Group II vs. Group III to Group IX; Where, * represents significant at <0.05,

** represents highly significant at p< 0.01, and
*** represents very significant at p<0.001. All values are compared with toxicant

Histopathology of the liver of various groups:

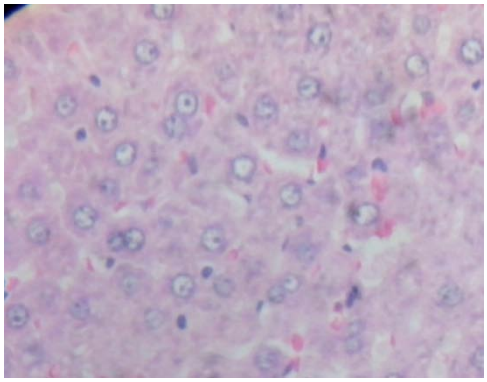

Fig. 1 : Group I

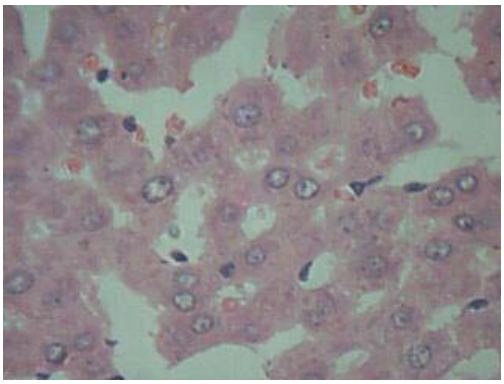

Fig. 2 : Group II

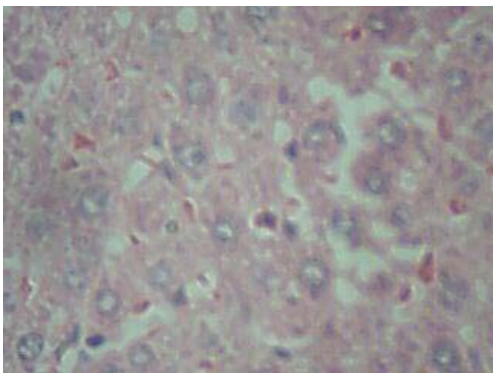

Fig. 3 : Group III

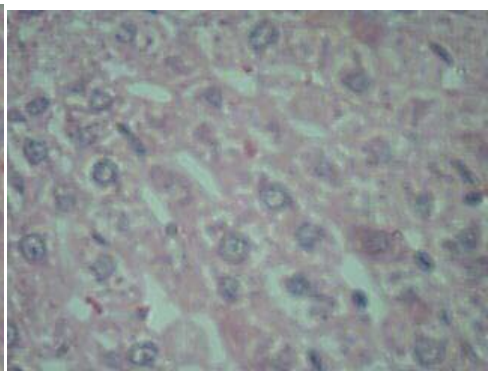

Fig. 4 : Group IV

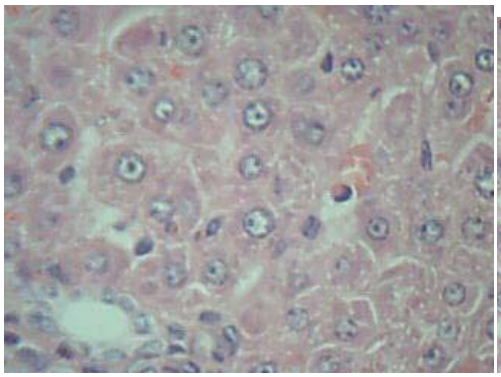

Fig. 5 : Group V

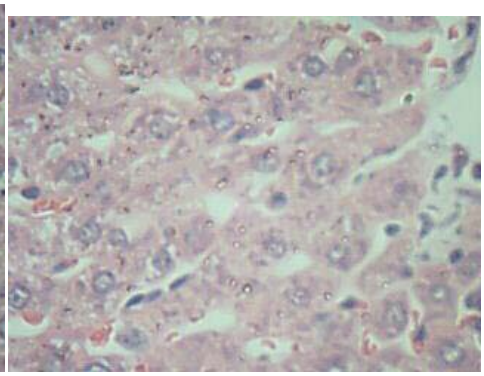

Fig. 6 : Group VI

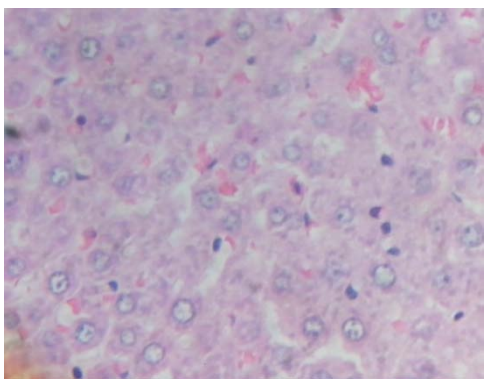

Fig. 7 : Group VII

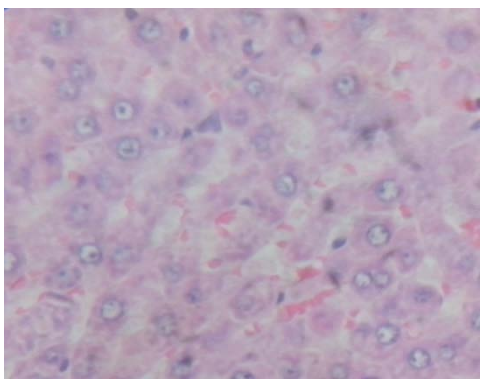

Fig. 8 : Group VIII

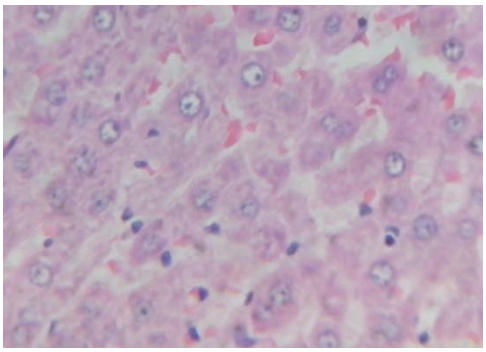

Fig. 9 : Group IX

IV. Discussion

The liver is a vital organ present in vertebrates and some other animals. The liver can be injured by many chemicals and drugs.[34] It is reported that more than 900 drugs have been implicated in causing liver injury [2] and it is the most common reason for a drug to be withdrawn from the market. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.[3, 4]. Statins are now among the most frequently prescribed medications and are currently used by about 25 million people worldwide. During hepatic damage, cellular enzyme like SGOT, SGPT, ALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase in concentration. [35]

In the previous study, it was reported that simvastatin caused oxidative stress mediated hepatotoxicity.[36] The most common clinical hepatic manifestation of statins is asymptomatic elevation in aminotransferases.[37-40] Elevations to.3 times upper limit of normal occur in 1% to 3%.[41]

In the present study, it shows that ethanolic extracts of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* significantly decreased the elevated serum marker enzymes and reversed the altered total protein to almost normal level. *Brassica oleracea L.var.capitata*(500mg/kg,) is shown to be more effective than ***Melia azedarach*** (500mg/kg,) *and Catharanthus Rosea* (500mg/kg,) while *Catharanthus Rosea* shows moderate activity than other extracts.It is reported that phenols are responsible for the variation in the antioxidant activity of the plant .[42] They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. [43, 44] Phenolic compounds are considered to be the most important antioxidative components of herbs and other plant materials. [45, 46]

Histological changes such as fatty changes in hepatocytes (steatosis) and perivenular fibrosis were observed in simvastatin intoxicated group. Ethanolic extracts of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* (300 mg/kg and 500mg/kg, p.o) significantly prevented these histological changes, further indicating their hepatoprotective activity. Although there is insufficient information to establish the mechanism of action of *Melia azedarach*,protection, this could be due to its antioxidant property of the phenolic compounds.

V. Conclusion

In conclusion, the results of present study demonstrate that *Melia azedarach* and *Brassica oleracea L.var.capitata* leaves extracts (300 mg/kg and 500mg/kg,) has potent hepatoprotective activity than *Catharanthus Rosea,* against simvastatin induced liver damage. Our study also implies that the hepatoprotective effects of *Melia azedarach* Linn, *Catharanthus Rosea* and *Brassica oleracea L.var.capitata* may be due to its antioxidant property of the phenolic compounds. Further investigation is in progress to determine the exact phytoconstituents responsible for hepataprotective effect.

Acknowledgments

My sincere thanks to my guide ***Dr. A Srinavasa Rao***, *Principal, Bhaskar Pharmacy College,* Hyderabad, INDIA, for rendering his suggestions and helping me in each and every step of completing this research work successfully.

References Références Referencias

1. Hai Zhong Huo, Bing Wang, Yong Kang Liang, Yong Yang Bao and Yan Gu. Hepatoprotective and Antioxidant Effects of Licorice Extract against CCl4-Induced Oxidative Damage in Rats, *Int. J. Mol. Sci.* 2011, *12*.
2. Friedman, Scott E.; Grendell, James H.; McQuaid, Kenneth R. (2003). *Current diagnosis & treatment in gastroenterology*. New York: Lang Medical Books/McGraw-Hill. pp. 664–679. ISBN 0-8385-1551-7
3. Friedman, Peter F. (2006). *GI/Liver Secrets: with STUDENT CONSULT Access*. Saint Louis: C.V. Mosby. ISBN 1-56053-618-7
4. Ostapowicz G, Fontana RJ, Schiødt FV, *et al.* (2002). "Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States". *Ann. Intern. Med.* **137** (12): 947–54. PMID 12484709.
5. Ricaurte B, Guirguis A, Taylor HC, Zabriskie D. Simvastatin-amiodarone interaction resulting in rhabdomyolysis, azotemia, and possible hepatotoxicity. Annals of Pharmacotherapy 2006;40(4): 753–7. [PubMed: 16537817]
6. Kanathur N, Mathai MG, Byrd RP Jr, Fields CL, Roy TM. Simvastatin-diltiazem drug interaction resulting in rhabdomyolysis and hepatitis. Tennessee Medicine 2001;94(9):339–41. [PubMed: 11550401].

7. Frei B, Kim M, Ames B. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. Proc Natl Acad Sci U SA 1990;87:4879-83 Stocker *et al.,* 1991*)*.
8. Stocker R, Bowry VW, Frei B. Ubiquinone-10 protects low density lipoprotein more efficiently against lipid peroxidation than does alphatocopherol. Proc Natl Acad Sci U S A 1991;88:1646-50.
9. Vaghasiya JD, Bhalodia YS, Manek RA, Gohil TA, Rathod SP. Oxidative stress mediated hepatotoxicity Produced by simvastatin. Pharmacologyonline 2008;3:495-503
10. Medhat, A., M. Shehata and S. Magder, 2002. Hepatitis C in a community in Upper Egypt: Risk factors for infection. Am. J. Trop. Med. Hyg., 66: 633-638.
11. Friedman, S.L., 2000. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J. Biol. Chem., 275: 2247-2250.
12. Fraschini F, Demartini G, Esposti D. Pharmacology of Silymarin. Clin Drug Inv 2002; 22: 51-65.
13. Mishra P., Uniyal G.C., and Sharma S., Pattern of diversity for morphological and alkaloid yield related trades among the periwinkle *Catharanthus roseus* accessions collected from in and around Indian Subcontinent, *Genetic Res Crop Evol.*, 2001, 48, 273-286.
14. Farnsworth N.R., Svoboda G.H., Blomster R.N., Antiviral activity of selected Catharanthus alkaloids, *J Pharm. Sci.*, 1968, 57, 2174-2175.
15. Svoboda G.H., Blake D.A., The phyto-chemistry and pharmacology of *Catharanthus roseus* (L.) G. Don. Inc. In: Taylor, W.J., Farnsworth, N.R. (eds.): The Catharanthusalkaloids. Marcel Decker, New York, 1975, 45-84.
16. P. C. Sharma, M.B. Yelne, T. J. Dennis, Data base on Medicinal plants used in Ayurveda, (Documentation and Publication Division, Central Council for Research in Ayurveda and Siddha, New Delhi, 2001) pp. 389-406.
17. C.P. Khare, Encyclopedia of Indian Medicinal Plants, (Springer, Germany) pp.305- 306.
18. P. Suhag, Merra and S.B. Kalidhar. Phytochemical investigation of *Melia azedarach* leaves. *J. Med. Aromatic Plant Sci.* **25(2):** 397-399 (2003).]\
19. Corpinella MC, Miranda M, Almiron WR, Ferrayoli CG, Almedia FL, Palacios SM. In vitro pediculicidal and ovicidal activity of an extract and oil from fruit of *Melia azedarach* L. *J Am Acad Dermatol* 2007; 56: 250-6.
20. Wandscheer CB, Duque JE, da silva MA, *et al.* Larvicidal action of ethanolic extracts from fruits endocarps of *Melia azedarach and Azadirachta indica* against the dengue mosquito Aedes Aegypti. *Toxicol* 2004; 44: 829-35.
21. Descalzo AM, Coto C. Inhibition of the pseudorabies virus (scis herpesvinyl) by an andvital agent isolated from the leaves of melia azedarach Rev. *Argent Microbial* 1989; 21: 133-40.
22. Choudhary DN, Singh JN, Verma SK, Singh BP. Antifertility effects of leaf extracts of some plants in male rats. *Indian J Exp Biol* 1990; 28: 714.
23. Goodwin JS, Brodwick M. J Am Diet Assoc 1996; 96:1027-1039.
24. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. *JAMA* 1996; 275:447-451.
25. Trease GE, Evans WC, A Text book of Pharmacognosy, 11th edition, Bailliere Tidall, London, 1978, 530.
26. Ecobichnon DJ. The Basis of Toxicity Testing. 2nd Ed. New York, NY: CRC Press; 1997.
27. Vaghasiya J, Rathod S, Bhalodia Y, Manek R, Malaviya S, Jivani N. Protective effect of polyherbal formulation on simvastatin hepatotoxicity in rats. J Young Pharmacists 2009;1:57-62.
28. Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
29. Walter K, Schutt C (1974). Acid and alkaline phoshatases in serum. In: Verlag Chemic Weinheim, In: Hans Ulrich Bergmeyer (Ed.), Method Enzymatic Anal. Academic Press Inc., New York, 2: 856-864.
30. Malloy HT, Evelyn KA (1937). The determination of bilirubin with the photoelectric colorimeter. J. Biol. Chem., 119: 481-490.
31. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folin-phenol reagent. J Biol Chem 1951; 193:265-275.
32. Luna LG (1999). Manual in histology and staining method. McGraw Hill: New York, p. 96.
33. Krajian AA (1963). Tissue cutting and staining. In: Frankel, S., Reitman,S.(Eds.), Gradwohl's Clinical Laboratory Method and Diagnosis. The CV. Mosby Co., Saint Louis, USA, p. 1639.
34. Leo MA, Arai M (1982). Hepatotoxicity of vitamin A and CCl_4. Gastroenterology, 82: 194-205.
35. Deb AC (1998). Fundamental of Biochemistry. 7th Ed. New Central Book Agency: Kolkata.
36. Vaghasiya JD, Bhalodia YS, Manek RA, Gohil TA, Rathod SP. Oxidative stress mediated hepatotoxicity Produced by simvastatin. Pharmacologyonline 2008;3:495-503.
37. Pasternal RC, Smith SC, Bairez-Merz C, Grundy SM, Cleeman JI, Len-fant C. ACC/AHA/NIHLBI clinical advisory on the use and safety of statins. Circulation 2002;106:1024-1028.
38. The Physician Desk Reference 2005, 59th Edition. Montvale, NJ: Thompson HealthCare.2005.

39. Farmer JA, Torre-Amione G. Comparative tolerability of the HMG-C0A reductase inhibitors. Drug Safety 2000;23:197-213.
40. Parra JL, Reddy KR. Hepatotoxicity of hypolipidemic drugs. Clin Liver Dis 2003;7:415-433.
41. Willis C. Maddrey, MD, Drug-Induced Hepatotoxicity J Clin Gastroenterol.Volume 39, Supp. 2, April 2005.
42. Cai Y, Luo Q, Sun M, Corke H, (2004). Antioxidant activity and phenolic compounds of 112 traditionalChinese medicinal plants associated with anticancer. *Life science*, 74: 2157-2184.
43. Pitchaon M, Suttajit M, Pongsawatmani R, (2007). Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. *Food Chem,* 100, 1409-1418.
44. Pokorny J, Yanishlieva N, Gordon M, (2001). Antioxidants in food, Practical Applications,Cambridge).Woodhead Publishing Limited, pp.1-3
45. Madsen HL, Nielsen BR, Bertelsen G, & Skibsted LH, (1996). Screening of antioxidative activity of spices.*Food Chemistry*, 57,331-337.
46. Pellegrini N, Simonetti P, Gardana C, Brenna O, (2000). Brighenti activity of Vini Novelli (Young red wines).*Journal of Agriculture and Food Chemistry*, 48, 732-735.

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