



HEPATOPROTECTIVE EFFECT OF *PLUMBAGO ZEYLANICA* ON PARACETAMOL INDUCED LIVER TOXICITY IN RATS

N. KANCHANA^{1*} AND A. MOHAMED SADIQ²

¹P.G and Research Department of Biochemistry, Jaya College of Arts and Science, Thiruninravur - 602 024. Tamilnadu. India, ²P.G and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, Vellore District - 632 506. Tamilnadu. India, Adhiparasakthi College of Arts and Science, G.B.Nagar, Kalavai - 632 506 Email: kanchi0402@yahoo.co.in

Received: 16 Oct 2010, Revised and Accepted: 20 Nov 2010

ABSTRACT

Petroleum ether extract of root of *Plumbago zeylanica* was investigated for hepatoprotective activity against paracetamol induced liver damage. Various biochemical parameters were studied to evaluate the hepatoprotective activity of ethanolic extract. In serum total bilirubin, total protein, aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, γ -Glutamyl transferase, Total Cholesterol and serum triglycerides were determined to assess the effect of the extract on the paracetamol induced hepatic damage. The study was also supported by histopathology of liver sections. Results of this study revealed that the markers in the animals treated with paracetamol recorded elevated concentration indicating severe hepatic damage by paracetamol, whereas the blood samples from the animals treated with petroleum ether extract of roots showed significant reduction in the serum markers indicating the effect of the plant extract in restoring the normal functional ability of the hepatocytes. The dosage of extract of plant roots used was 300 mg/kg bodyweight of rat. The present study reveals that the petroleum ether root extract of *Plumbago zeylanica* could afford a significant protection against paracetamol-induced hepatocellular injury.

Keywords: *Plumbago zeylanica*, Petroleum ether extract of roots, Hepatoprotective activity, Paracetamol damage.

INTRODUCTION

Herbs play a major role in the management of various liver disorders along with other system associated diseases. Liver is a key organ regulating homeostasis within the body by various functions. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity is one of very common ailment resulting into serious debilities ranging from severe metabolic disorders to even mortality¹. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity²⁻⁴.

Among the medicinal plants, *Plumbago zeylanica* (Family – Plumbaginaceae) (known as Chitrak) is a useful Indian medicinal plant which has been credited with therapeutic properties to treat several diseases. The root is blackish brown, knotted with horny fracture. Its leaves are 3 cm or so wide and about 5 cm long⁵. The root of the plant contains several bioactive chemical constituents which include plumbagin, 3-chloroplumbagin, droserone, chitranone, zeylanone, isozeylanone, plumbazeylone, coumarin, elliptinone, triterpenoids, β -sitosterol, maritinone, 2-methylnaphthazarin and anthroquinones⁶. Plumbagin is a naphthoquinone and is a major component constituting about 0.03% of dry weight of the roots and is considered as the active ingredient responsible for therapeutic effects⁷. Chitrak has been used for liver problems, loose motions, chronic skin diseases, gynecological practices, dysentery, anemia and obesity. The active component plumbagin increased the fecal excretion of cholesterol and phospholipids⁸. It prevented the accumulation of cholesterol and triglycerides in liver⁹. The present study was undertaken to study the possible hepatoprotective role of petroleum ether extract of roots of *Plumbago zeylanica*.

Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates^{10, 11}. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450¹² to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI)¹³. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid¹⁴. However, when the rate of NAPQI formation exceeds the rate

of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. Silymarin is marketed as one of the standard hepatoprotective herbal formulation.

MATERIALS AND METHODS

Plant materials

The roots of *Plumbago zeylanica* was collected from Adhiparasakthi Agricultural College Medicinal Park in Kalavai. The plant was identified at the Herbarium of Botany Directorate in Adhiparasakthi Agricultural College. A voucher specimen (No: Pz02) was deposited in the Botany Department of Adhiparasakthi Agricultural College, Kalavai, Tamilnadu, India.

Preparation of plant extract

The fresh dried powdered roots of *Plumbago zeylanica* were extracted (soxhlet) with petroleum ether three times at 60-80°C. All the extracts were mixed and evaporated by rotavapor. The concentrate was dissolved in minimum amount of distilled petroleum ether and the crystalline compound with a yield of 0.03% was obtained. The purity of this preparation was checked by thin layer chromatography (TLC) and gas liquid chromatography (GLC).

Chemicals

Paracetamol was purchased from, CIPLA LTD., Vill. Juddikalan, Baddi, H.P. Silymarin was supplied by Panacea Biotech Ltd, New Delhi. All other chemicals and other biochemicals used in the experiments were of analytical grade from different firms. The organic solvents were distilled before use.

Animals

Wistar Albino rats of either sex weighing between 100-200 g were used for this purpose. The animals were housed in polypropylene cages and maintained at $24 \pm 2^\circ$ under 12h light dark cycle and were fed *ad libitum* with standard pellet diet and had free access to water maintenance and use of animals as per the experiment was approved by the institutional Animal Ethics Committee.

Experimental designs

The animals were divided into 4 groups of six rats each. Group I animals served as normal control and received distilled water for

seven days. Group II animals orally received paracetamol (400 mg/kg body weight) for seven days. Group III animals received 200 mg/kg body weight of standard drug silymarin and 400 mg/kg body weight of paracetamol for seven days and served as standard control. Group IV animals received 400 mg/kg body weight of paracetamol dissolved in glucose water orally along with 300 mg/kg body weight of petroleum ether extract of roots of *Plumbago zeylanica* for seven days orally.

Sample collection

Animals of all the groups were sacrificed by cervical decapitation on eighth day. Blood sample of each group was collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37^o.

Evaluation of effect on biochemical variables

The clear serum obtained after centrifugation was used for the estimation of serum alanine amino transferase, serum aspartate amino transferase¹⁵, alkaline phosphatase¹⁶, gamma-glutamyl transferase¹⁷, Lactate dehydrogenase¹⁸, serum protein¹⁹, serum bilirubin²⁰, cholesterol²¹ and triglyceride²².

Histopathology study

Liver is dissected out and the liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique. The sections were taken at 5 μ thickness processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes.

Statistical Analysis

Results of biochemical estimations were reported as mean \pm SD of

six animals in each group. The data were subjected to one way ANOVA using SPSS 12.0 version (SPSS, Cary, NC, USA) followed by Bonferroni's multiple comparison tests (BMCT). The P-Value was <0.01 were considered statistically significant.

RESULTS

Effect of petroleum ether root extract of *Plumbago zeylanica* on paracetamol induced liver injury in rats with reference to biochemical changes in serum are given in Table 1 and 2.

Histological profile of animals is depicted in Figure 1,2,3,4. At the end of seven days treatment blood samples of paracetamol treated animals showed significant increase in the levels of total bilirubin, alanine amino transferase, aspartate amino transferase, alkaline phosphatase, lactate dehydrogenase, γ -glutamyl transferase, cholesterol and triglyceride compared to the normal control group, but the total protein level decreased reflecting the liver injury caused by paracetamol; whereas blood samples from the animals treated with root extract of *Plumbago zeylanica* at the dose 300 mg/kg body weight showed significant decrease in the levels of serum markers and significant increase in the total protein to the near normal value which are comparable to the values registered in the standard drug treated group of animals, indicating the protection of hepatic cells against paracetamol damage

DISCUSSION

Liver is largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification²³. Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases²⁴. Paracetamol being a drug capable of causing liver disorders if overdoses are consumed. The covalent binding of N-acetyl-P-benzoquinoneimine, an oxidation product of paracetamol, to sulphhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier^{25,26}.

Table 1: The effect of body weight and serum protein in normal, *Plumbago zeylanica* treated, Paracetamol induced hepatic damage, *Plumbago zeylanica* treated hepatic damage rats and Silymarin treated hepatic damage groups rats

Groups	Body weight(g)		Serum protein (g %)
	Initial (g)	Final (g)	
Normal	183	190	6.32 \pm 0.92**
Paracetamol induced hepatic damage	158	150	4.16 \pm 1.00**
Silymarin treated hepatic damage	173	180	5.85 \pm 1.19
<i>Plumbago zeylanica</i> treated hepatic damage	153	160	6.12 \pm 0.56**

Values are expressed as mean \pm SD for six animals in each group.

*Level of significance $p < 0.05$; **Level of significance $p < 0.01$

The paracetamol-induced liver damage was treated with *Plumbago zeylanica* for seven days continuously. The following observations were obtained. Table 1. represents the bodyweight of the different groups of rats. The body weight of Paracetamol induced group II rats has been reduced comparing with group I. After 7-days

administration of silymarin and *Plumbago zeylanica* on group III and group IV rats has gained the body weight almost to near normal level. The gain in body weight of group IV is compared with group III and it may be due to the regeneration of liver cells activity to near normal after the herbal treatment.

Table 2: The levels of serum bilirubin, serum cholesterol, and serum TG in normal, *Plumbago zeylanica* treated, Paracetamol induced hepatic damage, *Plumbago zeylanica* treated hepatic damage rats and Silymarin treated hepatic damage groups rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	LDH (IU/L)	γ - GT (IU/L)
Normal	92.35 \pm 2.23*	58.33 \pm 7.98*	73.67 \pm 6.41*	110.21 \pm 7.16*	2.02 \pm 0.09*
Paracetamol induced hepatic damage	159.98 \pm 13.23*	169.93 \pm 8.47*	131.80 \pm 12.30*	193.51 \pm 10.78*	4.82 \pm 1.10*
Silymarin treated hepatic damage	101.08 \pm 9.82**	68.83 \pm 7.65**	74.11 \pm .35**	116.38 \pm .58**	2.07 \pm 0.85**
<i>Plumbago zeylanica</i> treated hepatic damage	106.00 \pm 8.03*	62.00 \pm .52**	84.12 \pm .66**	98.69 \pm 13.08*	2.43 \pm 0.45**

Values are expressed as mean \pm SD for six animals in each group.

*Level of significance $p < 0.05$; **Level of significance $p < 0.01$

AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase, LDH = Lactate dehydrogenase, γ -GT = Gamma glutamyl transferase, TG = Triglyceride, IU/L = International units/litre,

Table 2 represents the changes in the activities of aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase γ -glutamyl transferase. In the assessment of liver damage by paracetamol the determination of enzyme levels such as aspartate transaminase and alanine transaminase is largely used. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver²⁷. Hepatocellular necrosis leads to high level of serum markers in the blood, among these, aspartate transaminase, alanine transaminase represents 90% of total enzyme and high level of alanine transaminase in the blood is better index of liver injury, but the elevated levels of enzymes are decreased to near normal levels after seven days treatment of *Plumbago zeylanica* indicates that it offered protection by preserving the structural integrity of the hepatocellular membrane against paracetamol.

Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure²⁸. Increased level was obtained after paracetamol administration and it was brought to near normal level by *Plumbago zeylanica* treatment.

Lactate dehydrogenase is localized in the cytoplasm of cells and thus is extruded into the serum when cells are damaged or necrotic. The measurement of total lactate dehydrogenase can be useful when only a specific organ, such as the liver, is known to be involved. Lactate dehydrogenase is increased in acute necrosis of the liver. Lactate dehydrogenase is a sensitive intracellular enzyme which increases in serum is also an indication of liver cell damage²⁹.

γ -glutamyl transferase is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ -glutamyl transferase is generally elevated as a result of liver disease, since γ -glutamyl transferase is a hepatic microsomal enzyme. Serum γ -glutamyl transferase is most useful in the diagnosis of liver diseases. Changes in γ -glutamyl transferase is parallel to those of amino transferases. The acute damage caused by paracetamol increased the γ -glutamyl transferase level but the same attains the normal after *Plumbago zeylanica* treatment due to its antioxidant activity.

Chronic administration of paracetamol produced a marked elevation of the serum levels of enzymes in treated animals when compared with that of the control group. Treatment with *Plumbago zeylanica* at a dose of 300 mg/kg significantly reduced the elevated levels of those enzymes. Treatment with *Plumbago zeylanica* decreased the serum levels of aspartate transaminase, alanine transaminase towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue

damage caused by paracetamol. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchymal cells. Effective control of alkaline phosphatase, lactate dehydrogenase, γ -glutamyl transferase levels points towards an early improvement in the secretory mechanism of the hepatic cell.

Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum proteins except for the γ globulins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. Albumin is decreased in chronic liver disease and is generally accompanied by an increase in the β and γ globulins as a result of production of IgG and IgM³⁰. Hypoproteinemia was observed after paracetamol ingestion but the trend turns towards normal after *Plumbago zeylanica* treatment.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes³¹. Administration of *Plumbago zeylanica* decreased the level of bilirubin and increased the level of protein suggesting that it offered protection.

Paracetamol seems to cause impairment in lipoprotein metabolism³² and also alterations in cholesterol metabolism. The levels of cholesterol and triglyceride were significantly increased in paracetamol treated rats, when compared to control, silymarin and *Plumbago zeylanica* treated rats. Elevation of tryglycerides level during paracetamol intoxication could be due to increased availability of free fatty acids, decreased hepatic release of lipoprotein and increased esterification of free fatty acids. Administration of *Plumbago zeylanica* significantly decreased serum lipid profile in paracetamol toxicity induced rats because of its hypolipidemic effects. *Plumbago zeylanica* supplementation enhanced esterification effect through hepatoprotective property by inhibiting the free radicals effect on liver cells.

Histological profile of control animals showed normal hepatocytes (fig.1). The section of the liver of the toxic control group of animals exhibited severe intense congestion, hydropic degeneration, pyknosis and occasional necrosis (fig.2). The liver section of the silymarin treated animals showed normal hepatic architecture with few fatty globules (fig.3). The liver section of the animals treated with root extract showed normal hepatic cords and absence of severe congestion, pyknosis and occasional necrosis (fig.4) indicating pronounced protection of hepatocytes by paracetamol induced hepatic damage.

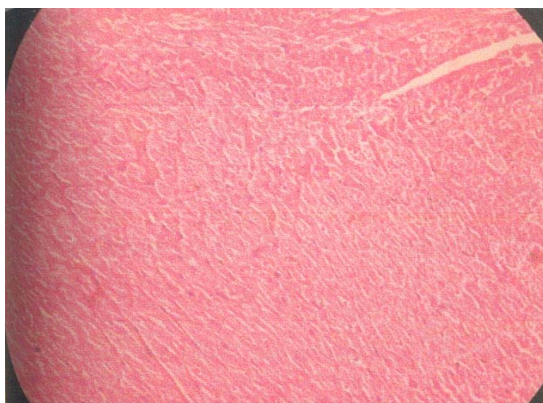


Fig. 1 (Normal)

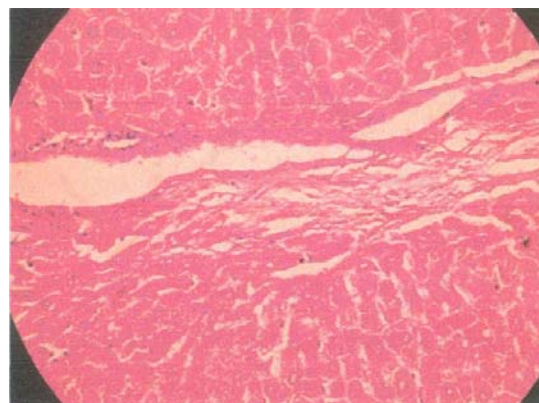


Fig. 2 (Paracetamol induced)

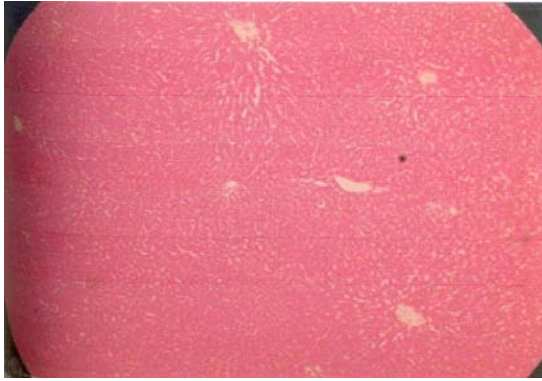


Fig.3 (Silymarin treated)

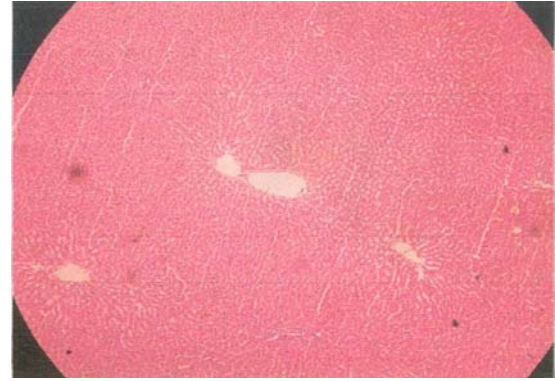


Fig.4 (Plumbago zeylanica treated)

Fig.1 Control Rat Liver H & E x100; Normal architecture of liver

Fig.2 PCM treated Rat Liver H & E x100; Severe congestion, hydropic degeneration, Pyknosis and occasional necrosis

Fig.3 PCM + Silymarin treated Rat Liver H & E x100; Near normal appearance of hepatocytes

Fig.4 PCM + *Plumbago zeylanica* treated Rat Liver H & E x100; Near normal appearance of hepatocytes (no severe congestion, Pyknosis and necrosis)

CONCLUSION

Several phytoconstituents viz., triterpenes, sterols, zeylonone of *Plumbago zeylanica* have been found effective in the hepatoprotection against paracetamol-induced hepatic toxicity. So results of this study demonstrated that the *Plumbago zeylanica* has significant protection on paracetamol-induced hepatotoxicity.

REFERENCES

- Patel RK, Patel MM, Patel MP, Kanzaria NR, Vaghela KR, Patel NJ. Hepatoprotective activity of *Moringa oleifera* Lam. Fruit on isolated rat hepatocytes. Phcog mag 2008;4:118-23.
- DeFeuids FV, Papadopoulos V, Drieu K. *Ginko biloba* extracts and cancer a research area in its infancy. Fundam Clin Pharamacol 2003;17:405-17.
- Banskota AH, Tezuka Y, Adnyana IK, Xiong Q, Hase K, Tran KQ, et al. Hepatoprotective effect of *Commibretum quadrangulare* and its constituents. Biol Pharm Bull 2000;23:456-60.
- Takeoka GR, Dao LT. Antioxidant constituent of almond (*Prunus dulcis* (Mill.) D.A.Webb) hulls. J Agri Food Chem 2003;51:496-501
- Puri HS. Rasayana: Ayurvedic herbs for longevity and rejuvenation. London: Taylor & Francis; 2003. p. 98-101.
- Gupta AA, Siddiqui IR, Singh J. A new anthraquinone glycoside from the roots of *Plumbago zeylanica*. Indian J Chem 2000;398:796-8.
- Gupta MM, Verma RK, Uniyal GC, Jain SP. Determination of Plumbagin by normal phase high performance liquid chromatography. J Chromat 1993;637:209-12.
- Purushothaman KK, Mohana KT Susan. Biological profile of plumbagin. Bulletin Medico Ethnobotanical Research 1985;6:177-88.
- Sharma I, Gusain D, Dixit VP. Hypolipidaemic and antiatherosclerotic effect of plumbagin in rabbits. Indian Journal Physiol Pharmacol 1991;35:10-4.
- Jollow DJ, Thorgeirsson SS, Potter WZ, Hashimoto M, Mitchell JR. Acetaminophen induced hepatic necrosis VI. Metabolic disposition of toxic and non-toxic doses of acetaminophen. Pharmacology 1974;12:251-71.
- Wong LT, Whitehouse LW, Solemonraj G, Paul CJ. Pathways of Acetaminophen conjugate in the mouse. Toxicity Lett 1981;9:145-51.
- Savides MC, Oehne FW. Acetaminophen and its toxicity. J App Toxicol 1983;3:95-111.
- Vermeulen NPE, Bessems JGM, Van de Streat R. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism based prevention. Drug Metab Rev 1992;24:367-407.
- Moore M, Thor H, Moore G, Nelson S, Moldeus P, Correnius S. The toxicity of acetaminophen and N-acetyl P-benzoquinoneimine in isolated hepatocytes is associated with thio depletion and increased cytosolic Ca²⁺. J Biol Chem 1985;260:13035-40.
- Reitzman S, Frankel S. A Colorimeter method for the determination of serum glutamic oxalo acetic acid, glutamic pyruvate transaminase. Am J Clin Pathol 1957;28:56-63.
- King EJ, Armstrong AR. Determination of serum and bile phosphatase activity. Canad Med Assoc J 1934;31:56-63.
- Rosalki SB, Rav D. Serum gamma - glutamyl transpeptidase activity in alcoholism. Clin Chem Acta 1972;39:41-7.
- King J. The hydrolase and alkaline phosphatase. In: practical clinical enzymology. London: Nostrand Co. Ltd; 1965. p. 191.
- Lowry OH, Rosebrough NJ, Farr AL, Randall. R.J. Protein measurement with Folin's Phenol reagent. J Biol Chem 1951;193:265-75.
- Malloy HT, Evelyn EA. The determination of bilirubin with photoelectric calorimeter. J Biol Chem 1937;119:481-5.
- Parekh AC, Jung DH. Cholesterol determination with ferric acetate-uranium acetate and sulfuric acid-ferrous sulfate reagents. Anal Chem 1970;42:1423-7.
- Gupta AA, Siddiqui IR, Singh J. A new anthraquinone glycoside from the roots of *Plumbago zeylanica*. Indian J Chem 2000;398:796-8.
- Larrey D. Drug induced liver disease. J Hepatol 2003;32:77-88.
- Watkins PB, Seef LB. Drug induced liver injury: Summary of a single topic research committee. Hepatology 2006;43:618-31.
- Handa SS, Sharma A. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against CCl₄. Ind J Med Res (B) 1990;92:276-83.
- Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen induced hepatic necrosis: (II) Role of covalent binding *in vivo*. J Pharmacol Exp Ther 1973;187:195-202.
- Drotman RB, Lawhorn GT. Serum enzymes are indicators of chemical induced liver damage. Drug Chem Toxicol 1978;1:163-71.
- Gaw A, Cowan RA, O'Reilly DSJ, Stewart, MJ Shepherd J. Clinical biochemistry - an illustrated color text. 1st ed. New York: Churchill Livingstone; 1999. p. 51-3.
- Kim KA, Lee WK, Kim JK, Seo MS, Lim D, Lee KH et al. Mechanism of refractory ceramic fibre- and rock wool-induced cytotoxicity in alveolar macrophages. Arch Occup Environ Health 2001;74:9-15.
- Kaplan LA, Pesce AJ. Clinical chemistry. 3rd ed. St. Louis, MO: Mosby; 1996. p. 517.
- Wolf A, Diez-Fernandez C, Trendelenburg CF, Prieto P, Hary S, Trammer WE. Paracetamol induced oxidative stress in rat hepatocytes. J Pharm Exp Ther 1997;280:1328-34.
- Kobashigania JA, Kasiska BL. Hyperlipidemia in solid organ transplantation. Transplantation 1997;63:333-8.