Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

HEPATOPROTECTIVE EFFECT OF CURCUMIN VERSUS SILYMARIN ON PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

***REHAM E. MASOUD**

**Lecturer of clinical pharmacology, Faculty of Medicine, PortSaid University, Egypt*

ABSTRACT

Paracetamol is widely used as analgesic-antipyretic leads to liver dysfunction. Silymarin has a hepatoprotective, immunomodulator, anti-inflammatory, antioxidant, and antifibrotic activities. Curcumin suppresses inflammation by reducing levels of inflammatory cytokines and has antioxidant effect. This study assessed the probable protective effect of curcumin against liver dysfunction and compare it to a hepatoprotective agent, Silymarin. The study groups included: normal control, paracetamol treated group (400mg/kg),silymarin (200mg/kg) + paracetamol treated group, curcumin (400mg/kg)+paracetamol treated group. After sacrifice ,plasmatic levels of liver function markers: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) of all groups, were determined. Bilirubin and total bilirubin were measured. Liver injury was assessed using histological studies. Samples of their livers were then used to determine glutathione level. There was a significant increase ($p < 0.05$)in liver markers in paracetamol treated group compared to normal and treated group. Curcumin treated group showed nonsignificant difference compared to Silymarin treated group. Curcumin treated group showed sidnificant increase in liver glutathione level. Both groups showed marked improvement in liver architecture as compared to paracetamol treated group.The available evidences in this study suggest that the complementary effects of silymarin or Curcmin proved to be capable of ameliorating probable-mediated hepatic oxidative damage and the probable mechanism is via antioxidative action.

KEY WORDS: ***Antioxidants; Hepatoprotective; glutathione; silymarin, curcumin.***

***REHAM E. MASOUD**

Lecturer of clinical pharmacology, Faculty of Medicine, PortSaid University, Egypt.

Received on : 03-02-2017
Revised and Accepted on : 07-03-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.p134-141

INTRODUCTION

Many patients require multiple drugs to treat various chronic diseases. The prescription of several drugs is linked to increase risk of drug side effects, one of these, hepatic injury. The liver is of a very important organ involved in metabolism and is continuously exposed to xenobiotics, environmental pollutants, and chemotherapeutic agents, since it is involved in detoxification and elimination of toxic substances[1]. Hepatic damage is associated with altered metabolic functions and it is still a severe health problem, since conventional drugs used in the treatment of liver diseases have serious adverse effects[2]. Paracetamol is widely used as analgesic-antipyretic drug and is considered remarkably safe drug when used at usual therapeutic doses. its toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450[3]. It is metabolized by sulfation and glucuronidation of the parahydroxyl group. paracetamol hepatotoxicity is caused by its reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion, a prerequisite for APAP-induced hepatotoxicity[4-5] . In overdoses, it is a potent hepatotoxin, producing fulminant hepatic and renal tubular necrosis, which can be lethal in human and animal. Several studies about protection against hepatotoxicity have been investigated to ameliorate the livers disorders treatment. Many formulations containing herbal extracts are used for regeneration of hepatic cells and for protection of the liver against damage[6] . Silymarin (SLM) is a lipophilic extract isolated from the seeds and fruits of Silybummarianum, a herbaceous plant belonging to the family Compositae and native to a narrow area of the Mediterranean. SLM is composed of several flavonolignans isomers (65–80%) with small amounts of flavonoids and fatty acids (20–35%) and other polyphenolic compounds[7-9]. The main isolated and structural active component of SLM is silybin, comprising about 33% of total SLM weight, and is clinically used[10-11] as hepatoprotector to treat liver injuries . Besides, other biological activities for SLM, such as hepatoprotective/hepatic regenerator, immunomodulator, anti-inflammatory, antioxidant, and antifibrotic activities were described[12-13] . There is evidence that SLM is effective in hepatic disease induced by different drugs[14] . Currently, SLM is used as a reference drug in the screening of new drugs hepatoprotective[9] .Curcumin attenuates oxidative stress by increasing the content of hepatic glutathione, leading to the reduction in the level of lipid hydroperoxide. Curcumin dramatically suppresses inflammation by reducing levels of inflammatory cytokines, including interferon-γ, tumor necrosis factor-α, and interleukin-6. Furthermore, curcumin inhibits HSC activation by elevating the level of PPARγ and reducing the abundance of platelet-derived growth factor, transforming growth factor-β, their receptors, and type I collagen [14].This study aimed to evaluate and compare the ameliorator property of silymarin and curcmin against N-acetyl-p-aminophenol (APAP- paracetamol)-induced injury in male rat at the biochemical, histological properties.

METHODS

Animals

Thirty-two male albino rats of body weight ranging from 170-200 g were included in the study. Rats were purchased from the Ophthalmic Research Institute animal house in Giza. Rats were kept under controlled laboratory setting, normal day/night cycle, temperature 23±2 ^{o}C and humidity ranging 45-55%. Rats were housed in polypropylene cages (8 per cage) with food and water given ad libitum. Rats were acclimatized for one week before the start of the study. Procedures involving animals were conducted according to ethical committee guidelines that comply with national and international laws and policies.

Study design

It is an experimental study that was carried out in the Pharmacology department, Faculty of Medicine, Suez Canal University. Rats were randomly assigned into four groups 8 rats each:

Group1: normal control group

Group2: Paracetamol treated group received APAP (400mg/kg)[15]

Group3: APAP + SLM (200 mg/kg)[16]

Group4:Paracetamol +curcumin treated group: APAP+Curcumin 400 mg/kg[14]

All animals were treated for 30 days

All administrations were done once daily for 30 days using oral intubator with ad libitum provision of food and water throughout the experimental period.

Chemicals

APAP was purchased from the Egyptian International Pharmaceutical Industries Company; silymarin was obtained from Sedeco Pharmaceutical Co-6-october City, Egypt. Curcmin were purchased from Sigma-Aldrich Chemicals Company (St. Louis, Mo, USA). Standard assay kits of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin and total protein were obtained from Sigma-Aldrich . All other reagents were of analytical grade.

Preparation of serum and excision of liver

Twenty-four hours after the last treatment, the rats were sacrificed. Blood was collected by cardiac puncture into centrifuge tubes and allowed to stay for 20 min before centrifuging at 3000 × g for 15 min . Serum was carefully aspirated and used for liver function tests. The liver was excised, cleaned of fat and sliced into two portions. A portion of the liver was homogenized in Tris–HCl buffer (0.05 mol/l Tris–HCl and 1.15% KCl, pH 7.4) for antioxidant analyses, while the other portion was fixed in saline formaldehyde solution for histological examination. Blood was drawn from the caudal vena cava and centrifuged to obtain serum for determination of glutathione (GSH),Liver GSH levels were measured using a modified Tietze assay as described by Jaeschke[17]

STATISTICAL ANALYSIS

Data were expressed as the mean ± SEM for each group. Results were statistically analyzed by using one-way variance analysis (ANOVA) followed by Tukey's

test. Differences were considered significant when P < 0.05. Statistical analyses were performed using SPSS (Version 15).

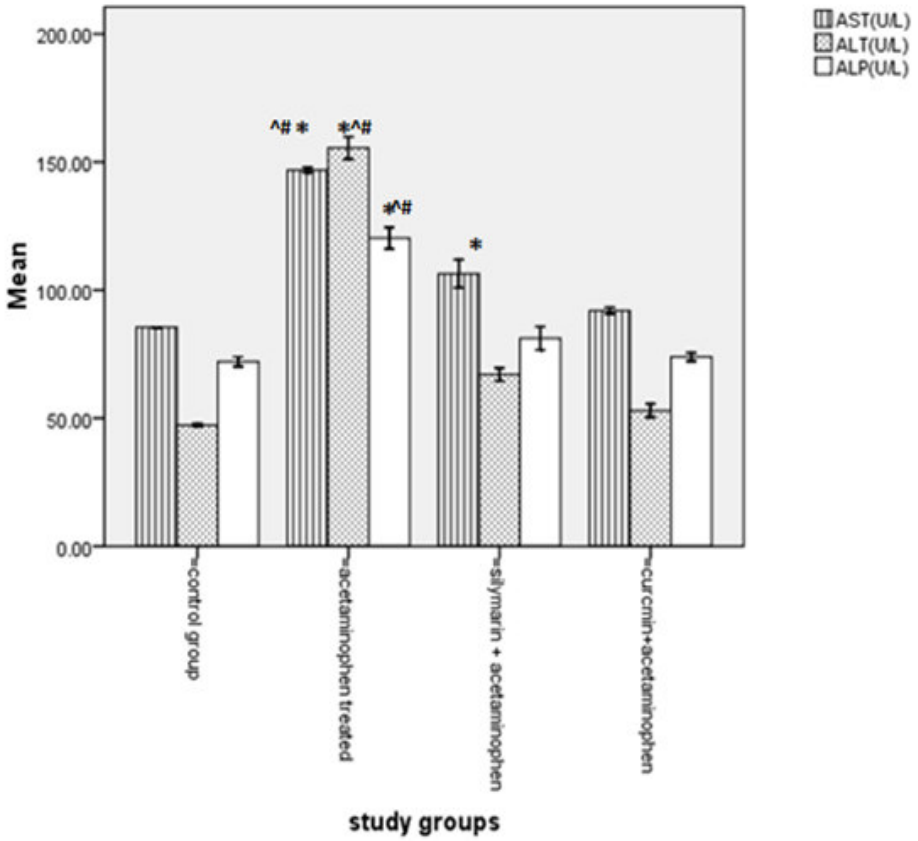
RESULTS

Effect of administered drugs on liver enzymes

There was significant increase in liver enzymes (AST,ALT,APL) in paracetamol treated group as compared to normal control group (P < 0.05), treatment with SIlimarin resulted in significant decrease in ALT and APL but AST remained significantly higher than normal but treatment with curcumin resulted in significant decrease in all liver enzymes P < 0.05 as shown in fig 1.

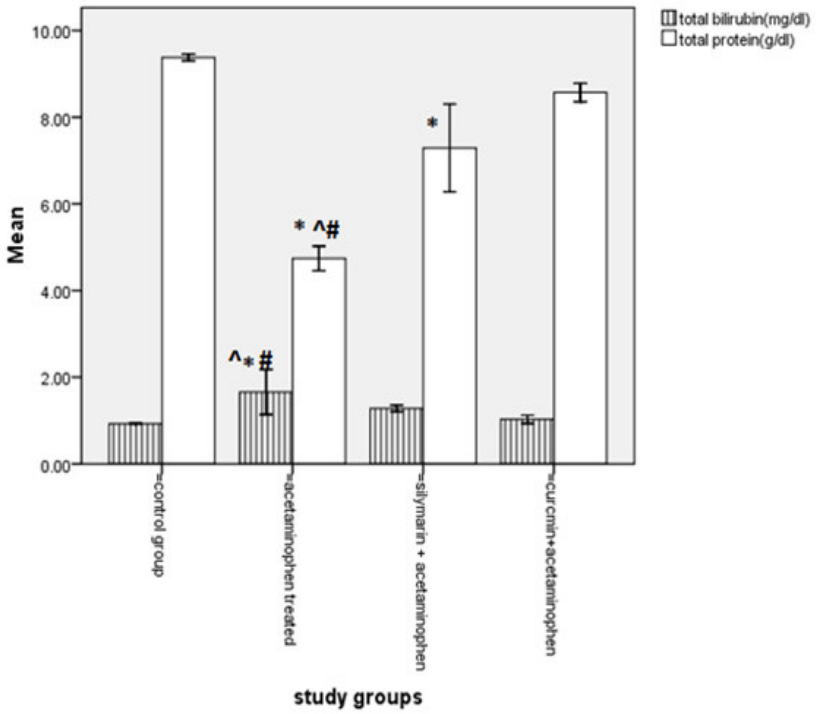
Effect on bilirubin and total protein

There was significant increase in bilirubin in paracetamol treated group and significant decrease in total protein compared to normal and treated groups (P < 0.05), group treated with Silymarin showed significant decrease of bilirubin however total protein level was significantly higher than normal yet lower than paracetamol treated group. In group treated with Curcmin there was significant decrease in bilirubin and significant increase in total protein (P < 0.05) as shown in figure 2.



***significant difference compared to normal group P < 0.05**
^ significant difference compared to Silymarin group P < 0.05
 # significant difference compared to Curcumin group P < 0.05

Figure 1
Mean ± SEM level of liver enzymes (U/L) in study groups after 30 days of treatment .

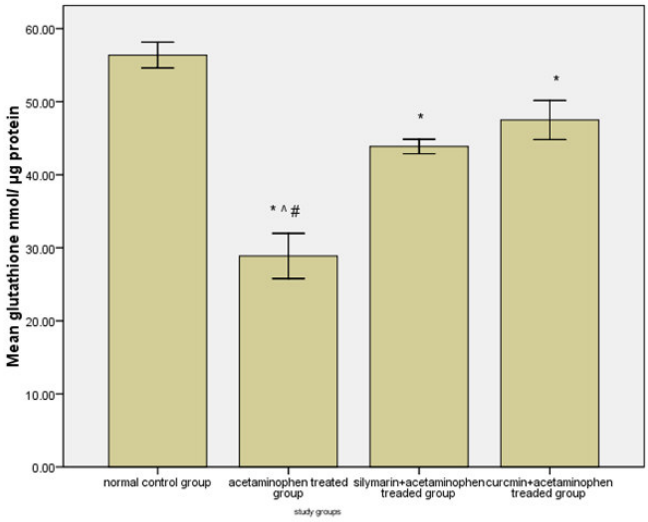


significant difference compared to normal group P < 0.05
^ significant difference compared to Silymarin group P < 0.05
 # significant difference compared to Curcumin group P < 0.05

Figure 2
level of bilirubin and total protein(mean ± SEM) in study groups after 30 days of treatment

Level of glutathione in liver tissue

There was significant decrease in glutathione level in paracetamol treated group as compared to normal and treated groups (P < 0.05),glutathione level in silymarin and curcumin treated group was significantly lower than normal but also significantly higher than paracetamol treated group indicating improvement of liver function as shown in figure 3.



significant difference compared to normal group P < 0.05
^ significant difference compared to Silymarin group P < 0.05
 # significant difference compared to Curcumin group P < 0.05

Figure3
level of glutathione in the liver of study groups (mean ± SEM)

Liver histopatholgy

In paracetamol treated group, there was distoration of liver architecture, there were also vaculation of hepatocytes, infiltration with inflammatory cells, all these changes were mildly improved in silymarin and curcumin treated groupsasshown in figure 4.

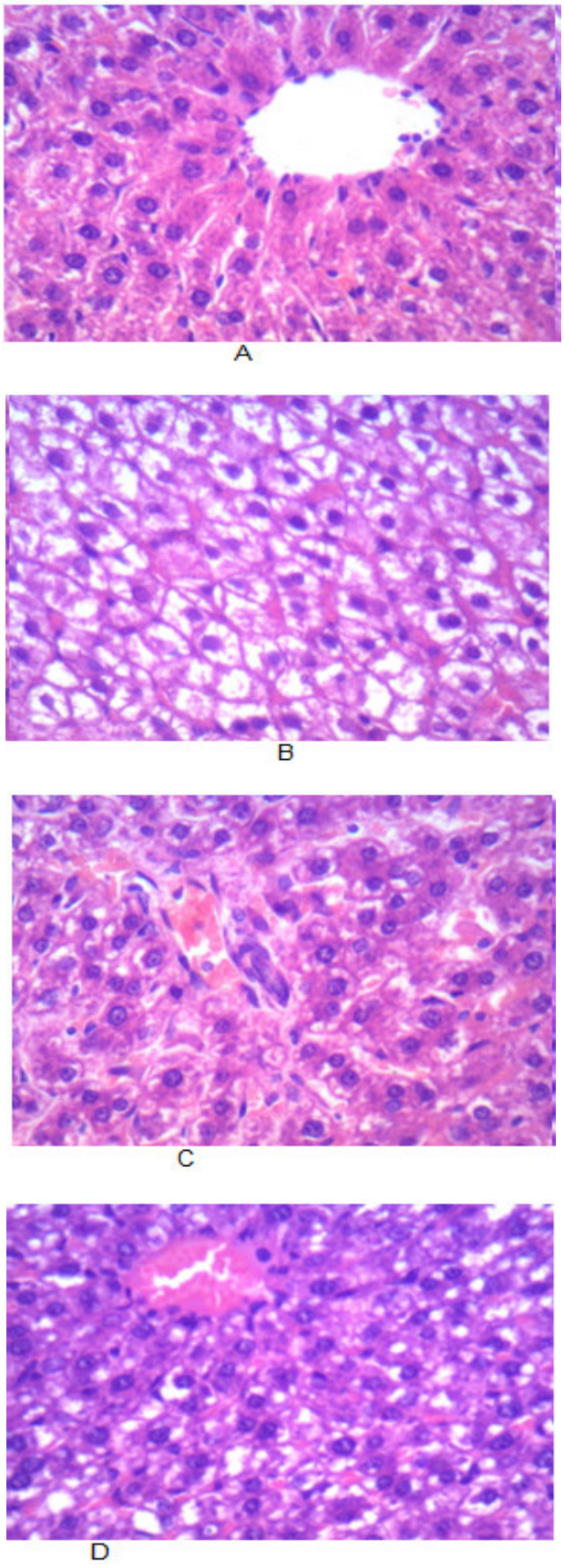


Figure 4

Photomicrographs of rat Liver sections in the different experimental groups stained with Haematoxylin& Eosin.

A: Rat Liver sections in control group revealed normal structure of hepatocytes. B: Rat Liver section in paracetamol treated group showed moderate to severe lose of liver architecture, disturbance of the hepatocytes, atrophied, and vacuolated hepatocytes . c: Liver sections inSilymarin treatedrevealed a moderate to mild degree of improvement in hepatocytes where a few vacuolated hepatocytes D: Liver section in curcumin treated group showed improvement in hepatocytes structure and arrangement.

DISCUSSION

Liver injuries induced by APAP is commonly used as a model for the screening of hepatoprotective activities of drugs, where free radicals and oxidative processes play an important role in hepatotoxicity[6]. A decrease in total serum protein after APAP treatment could be associated with the decrease in the number of hepatocytes, which in turn may result in the decreased hepatic capacity to synthesize protein and, consequently, decrease liver weight [18-19]. Hepatotoxic drugs, such as APAP, are known to cause marked elevation in serum level of enzymes, such as ALT, AST, ALP, and bilirubin, indicating significant hepatocellular injury[20] . When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver injury[21-22]. The liver activity functional is altered, since ALT, AST, γ-GT, and ALP levels are increased in SHR, but not for glucose levels[23] . Generally, there is a raised activity of serum transaminases in intoxicated rats , as observed in the present study. However, ALT and AST levels were increased but this is enough to be attributed to the damaged structural integrity of the liver because transaminases are cytoplasmic enzymes in nature and are released into the circulation after cellular damage[24]. Silymarin has hepatoprotective properties and is used in treatment of various liver diseases[25] . Various studies indicate that Silymarin exhibits strong antioxidant activity[26] and shows protective effects against hepatic toxicity induced by a wide variety of agents by inhibiting lipid peroxidation[27] . Higher total phenolic content has been known to contribute to the antioxidant activity of extracts[29] , while antioxidant activity has also been linked to the hepatoprotective effect of some extracts[29] . These findings corroborate with our results on the ability of SLM to exert a hepatoprotective activity.In this study, SLM treatment restore to normal values the levels of ALT, AST in all animals treated with APAP,. The reduced concentrations of these enzymes as a result of SLM administration might probably be, in part, due to the presence of chemical constituents in the extract [30]. The plasmatic reduction of these marker enzymes, to return to near normal values, would be owing to the antihepatotoxic effect of SLM.The decrease in AST and ALP activities supports the hepatoprotective effects of curcumin, consistent with the findings that curcumin modulated the increased activity of marker enzymes and plasma lipid levels in nicotine-treated rats[31] . The type of live rinjury is determined by measuring the presence of hepatocellular enzymes in liver like SGOT, SGPT and ALP. The increased levels of these enzymes indicate mitochondrial damage and cell membrane damage[32].The depletion of GSH is suggestive of a deleterious effect on the antioxidant defense in the liver of rats. It is also consistent with the generation of oxidative stress, thereby reenforcing the role of GSH in molecular protective mechanisms that modulate cellular responses to toxic chemicals. The present study showed that administration of curcumin improved the GSH levels in rats. This finding is similar to the data reported by Piper et al.[33] , who indicated an increase of the GSH level corresponding to curcumin dosage in rats fed curcumin, at doses up to 500 mg/kg body weight daily for 14 days.Histopathologic studies supported the evidence of biochemical parameters analysed in this study. Histological analyses of rat liver treated with APAP showed significant hepatotoxicity, characterized by inflammatory hepatic tissues, including the presence of moderate infiltration of neutrophils. There was extensive infiltration of inflammatory cells around the central vein and loss of cellular boundaries in all groups, after hepatotoxicity induced by APAP[34] . These histologic alternations in structure of the liver were improved by treatment with silymarin or curcumin.

CONCLUSION

This study concluded that curcumin was comparable to silymarin in prevention and improvement of liver dysfunction caused by paracetamol thus we recommend using it during chronic use of paracetamol to protect against liver dysfunction.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Gu X., Manautou J. E. Molecular mechanisms underlying chemical liver injury. Expert Reviews in Molecular Medicine. 2012;(14):676–687.
2. Hayden M. R., Sowers J. R. Treating hypertension while protecting the vulnerable islet in the cardiometabolic syndrome. Journal of the American Society of Hypertension. 2008;2(4):239–266
3. Plante G. E. Vascular response to stress in health and disease. Metabolism: Clinical and Experimental. 2002;51(6):25–30.
4. Mambelli E. Arterial hypertension in dialysis: up to what point should it be corrected? It depends. GiornaleItaliano di Nefrologia. 2011;28(3):261–265.
5. Das J., Ghosh J., Manna P., Sil P. C. Acetaminophen induced acute liver failure via oxidative stress and JNK activation: protective role of taurine by the suppression of cytochrome P450 2E1. Free Radical Research. 2010;44(3):340–355.
6. Aubert J., Begriche K., Delannoy M., et al. Differences in early acetaminophen hepatotoxicity between obese ob/ob and db/db mice. Journal of Pharmacology and Experimental Therapeutics. 2012;342(3):676–687.
7. Zhang L., Gavin T., Geohagen B. C., Liu Q., Downey K. J., LoPachin R. M. Protective properties of 2-acetylcyclopentanone in a mouse model of acetaminophen hepatotoxicity. Journal of Pharmacology and Experimental Therapeutics. 2013;346(2):259–269.
8. Jaeschke H., Williams C. D., McGill M. R., Xie Y., Ramachandran A. Models of drug-induced liver injury for evaluation of phytotherapeutics and other natural products. Food and Chemical Toxicology. 2013;55:279–289.
9. Kim N.-C., Graf T. N., Sparacino C. M., Wani M. C., Wall M. E. Complete isolation and characterization of silybins and isosilybins from milk thistle (Silybummarianum) Organic & Biomolecular Chemistry. 2003;1(10):1684–1689.
10. Lee J. I., Hsu B. H., Wu D., Barrett J. S. Separation and characterization of silybin, isosilybin, silydianin and silychristin in milk thistle extract by liquid chromatography-electrospray tandem mass spectrometry. Journal of Chromatography A. 2006;1116(1-2):57–68.
11. Elmowafy M., Viitala T., Ibrahim H. M., et al. Silymarin loaded liposomes for hepatic targeting: in vitro evaluation and HepG2 drug uptake. European Journal of Pharmaceutical Sciences. 2013;50(2):161–171.
12. Pradhan S. C., Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. Indian Journal of Medical Research. 2006;124(5):491–504.
13. Fehér J., Lengyel G. Silymarin in the prevention and treatment of liver diseases and primary liver cancer. Current Pharmaceutical Biotechnology. 2012;13(1):210–217.
14. Yumei Fu, Shizhong Zheng, Jianguo Lin, Jan Ryerse and Anping Chen. Curcumin Protects the Rat Liver from CCl4-Caused Injury and Fibrogenesis by Attenuating Oxidative Stress and Suppressing Inflammation Molecular Pharmacology February 2008, 73 (2) 399-409
15. 15.Kanchana, N., Mohamed, A.S., 2011. Hepatoprotective effect of Plumbagozeylanica on paracetamol induced liver toxicity in rats. Int. J. Pharm. Pharm. Sci. 3, 151-154.
16. Quan J., Yin X., Xu H. Boschniakiarossica prevents the carbon tetrachloride-induced hepatotoxicity in rat. Experimental and Toxicologic Pathology. 2011;63(1-2):53–59.
17. Jaeschke, H. (1990). Glutathione disulfide formation and oxidant stress during acetaminophen-induced hepatotoxicity in mice in vivo: The protective effect of allopurinol. J. Pharmacol. Exp. Ther. 255, 935–941.
18. Bhadauria M. Dose-dependent hepatoprotective effect of emodin against acetaminophen-induced acute damage in rats. Experimental and Toxicologic Pathology. 2010;62(6):627–635.
19. Oyagbemi A. A., Odetola A. A. Hepatoprotective effects of ethanolic extract of Cnidoscolusaconitifolius on paracetamol-induced hepatic damage in rats. Pakistan Journal of Biological Sciences. 2010;13(4):164–169.
20. Green T. J., Sivilotti M. L. A., Langmann C., et al. When do the aminotransferases rise after acute acetaminophen overdose. Clinical Toxicology. 2010;48(8):787–792.
21. Alkiyumi S. S., Abdullah M. A., Alrashdi A. S., Salama S. M., Abdelwahab S. I., Hadi A. H. A. Ipomoea aquatica extract shows protective action against thioacetamide-induced hepatotoxicity. Molecules. 2012;17(5):6146–6155.
22. Bell L. N., Vuppalanchi R., Watkins P. B., et al. Serum proteomic profiling in patients with drug-induced liver injury. Alimentary Pharmacology and Therapeutics. 2012;35(5):600–612.
23. Binda D., Nicod L., Viollon-Abadie C., et al. Strain difference (WKY, SPRD) in the hepatic antioxidant status in rat and effect of hypertension (SHR, DOCA). Ex vivo and in vitro data. Molecular and Cellular Biochemistry. 2001;218(1-2):139–146.
24. Kelava T., Ćavar I., Vukojević K., Saraga-Babić M., Čulo F. The effect of glucagon and cyclic adenosine monophosphate on acute liver damage induced by acetaminophen. Histology and Histopathology. 2013;28(2):245–255
25. Elmowafy M., Viitala T., Ibrahim H. M., et al. Silymarin loaded liposomes for hepatic targeting: in vitro evaluation and HepG2 drug uptake. European Journal of Pharmaceutical Sciences. 2013;50(2):161–171.
26. Simeonova R., Vitcheva V., Kondeva-Burdina M., Krasteva I., Manov V., Mitcheva M. Hepatoprotective and antioxidant effects of saponarin, isolated from Gypsophila trichotoma wend. on paracetamol-induced liver damage in rats. BioMed Research International.
27. Bosisio E., Benelli C., Pirola O. Effect of the flavanolignans of Silybummarianum L. on lipid

peroxidation in rat liver microsomes and freshly isolated hepatocytes. Pharmacological Research. 1992;25

28. Yuan L., Gu X., Yin Z., Kang W. Antioxidant activities in vitro and hepatoprotective effects of Nelumbonucifera leaves in vivo. African Journal of Traditional, Complementary and Alternative Medicines. 2014;11(3):85–91.
29. Gu F., Gu X., Xu Q., Kang W. Antioxidant activity in vitro and hepatoprotective effect of Phlomismaximowiczii in vivo. African Journal of Traditional, Complementary, and Alternative Medicines. 2014;11(3):46–52.
30. Cordero-Pérez P., Torres-González L., Aguirre-Garza M., et al. Hepatoprotective effect of commercial herbal extracts on carbon tetrachloride-induced liver damage in Wistar rats. Pharmacognosy Research. 2013;5(3):150–156.
31. Kalpana C., Rajasekharan K., and Menon V., “Modulatory effects of curcumin and curcumin analog on circulatory lipid profiles during nicotine-induced toxicity in Wistar rats,” Journal of Medicinal Food, vol. 8, no. 2, pp. 246–250, 2005.
32. Tejaswi C,Sesh k, Shubhangi H,et al., Hepatoprotective effect of polyunsaturated fatty acids against repeated subacute Acetaminophen dosing in rats. Int J Pharm Bio Sci 2013 Apr; 4(2): (P) 286 - 295
33. Piper J, Singhal S., M.Salameh,et al., “Mechanisms of anticarcinogenic properties of curcumin: the effect of curcumin on glutathione linked detoxification enzymes in rat liver,” International Journal of Biochemistry and Cell Biology, vol. 30, no. 4, pp. 445–456, 1998
34. Singhal R., Ganey P. E., Roth R. A. Complement activation in acetaminophen-induced liver injury in mice. Journal of Pharmacology and Experimental Therapeutics. 2012;341(2):377–385.

Reviewers of this article

Mona Hussin

Assistant Prof of Physiology,Head Of Physiology Dept,Faculty of Medicine,Portsaid University Egypt.

Mr. Anubrata Paul M.Sc. Biotech (Research)

Department of Biotechnology, Natural Products Research Laboratory, Centre for Drug Design Discovery & Development (C-4D) , SRM University, Delhi-NCR,Sonepat.

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript