



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

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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 significant 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 Curcumin 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.*



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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 substances¹. 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 effects². 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 P450³. 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 hepatotoxicity⁴⁻⁵. 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 damage⁶. Silymarin (SLM) is a lipophilic extract isolated from the seeds and fruits of *Silybum marianum*, 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 compounds⁷⁻⁹. The main isolated and structural active component of SLM is silybin, comprising about 33% of total SLM weight, and is clinically used¹⁰⁻¹¹ 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 described¹²⁻¹³. There is evidence that SLM is effective in hepatic disease induced by different drugs¹⁴. Currently, SLM is used as a reference drug in the screening of new drugs hepatoprotective⁹. 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 PPAR γ and reducing the abundance of platelet-derived growth factor, transforming growth factor- β , their receptors, and type I collagen¹⁴. This study aimed to evaluate and compare the ameliorator property of silymarin and curcumin 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 °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/kg¹⁴

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. Curcumin 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 $3000 \times g$ 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 Jaeschke¹⁷

STATISTICAL ANALYSIS

Data were expressed as the mean \pm 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).

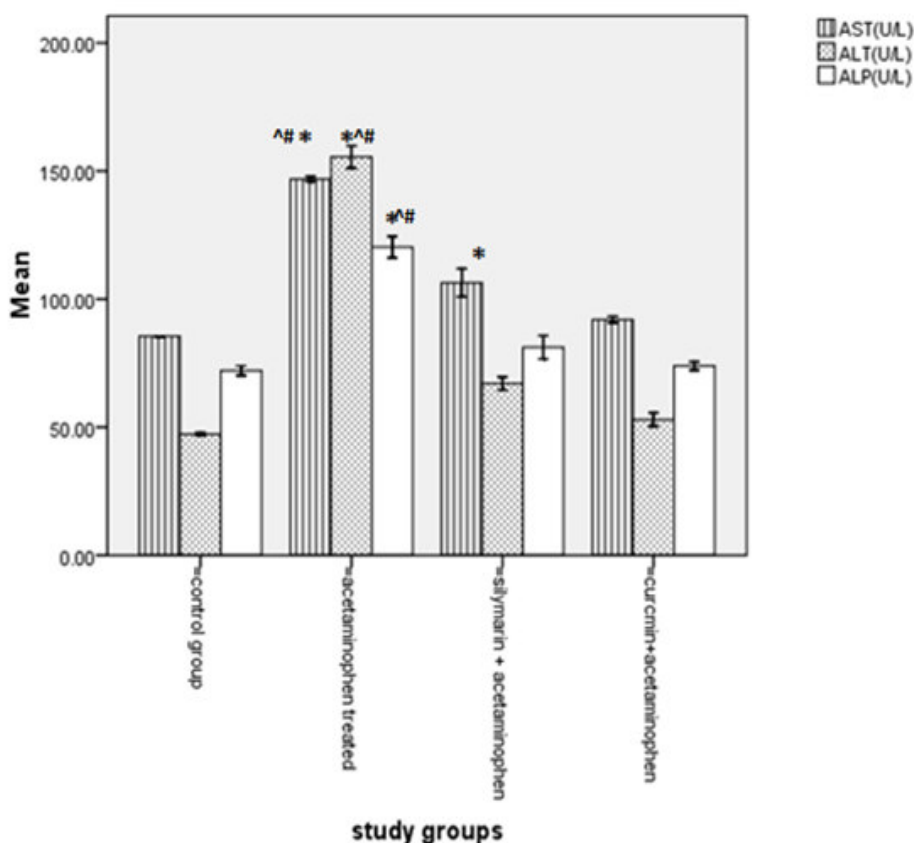
RESULTS

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 Silymarin 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.

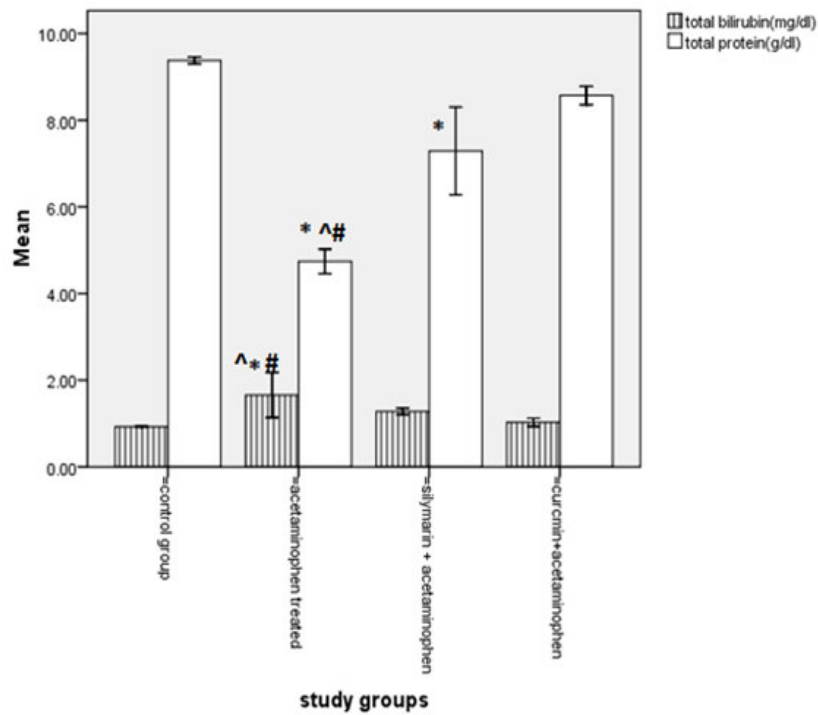
Effect on bilirubin and total protein

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 Curcumin 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 \pm SEM level of liver enzymes (U/L) in study groups after 30 days of treatment .



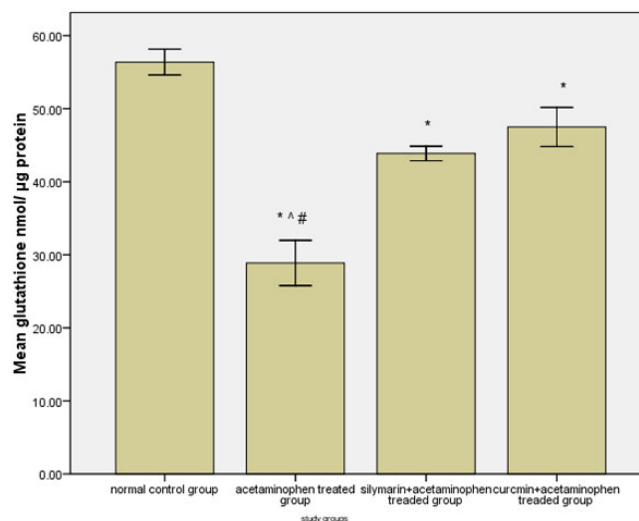
*significant difference compared to normal group $P < 0.05$
 ^ significant difference compared to Silymarin group $P < 0.05$
 # significant difference compared to Curcumin group $P < 0.05$

Figure 2
 level of bilirubin and total protein(mean \pm 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.



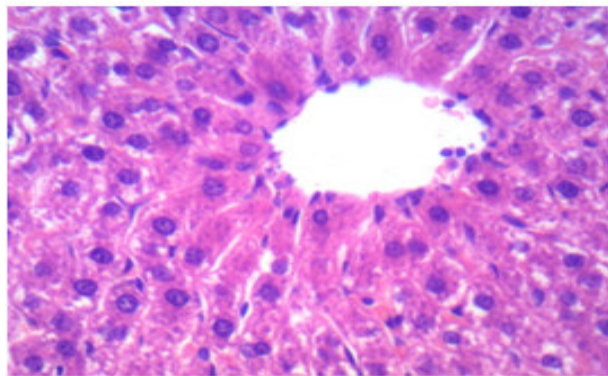
*significant difference compared to normal group $P < 0.05$
 ^ significant difference compared to Silymarin group $P < 0.05$
 # significant difference compared to Curcumin group $P < 0.05$

Figure 3
 level of glutathione in the liver of study groups (mean \pm SEM)

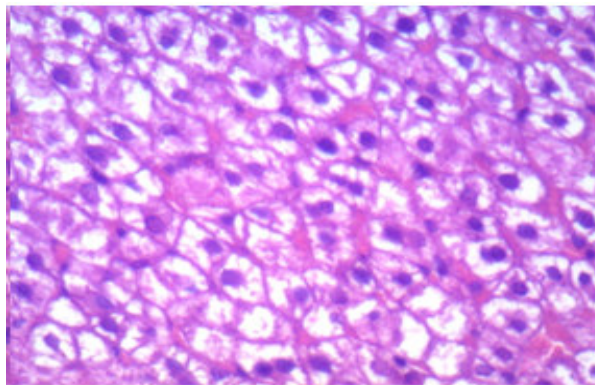
Liver histopathology

In paracetamol treated group, there was distortion of liver architecture, there were also vacuolation of

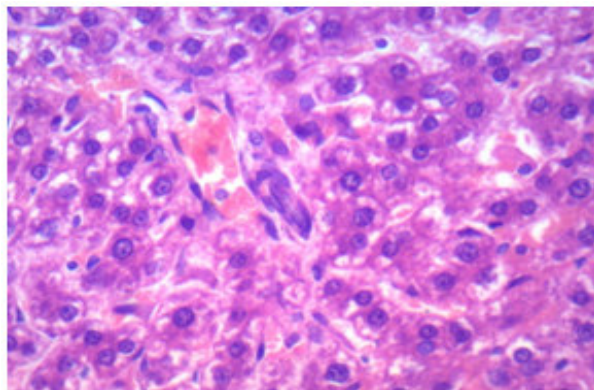
hepatocytes, infiltration with inflammatory cells, all these changes were mildly improved in silymarin and curcumin treated groups as shown in figure 4.



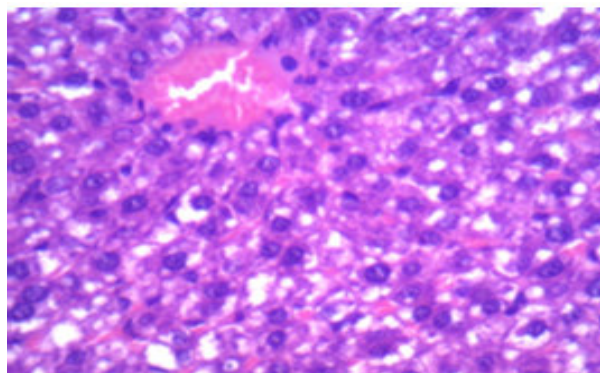
A



B



C



D

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 loss of liver architecture, disturbance of the hepatocytes, atrophied, and vacuolated hepatocytes. C: Liver sections in Silymarin treated revealed 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 hepatotoxicity⁶. 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 weight¹⁸⁻¹⁹. 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 injury²⁰. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver injury²¹⁻²². The liver activity functional is altered, since ALT, AST, γ -GT, and ALP levels are increased in SHR, but not for glucose levels²³. 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 damage²⁴. Silymarin has hepatoprotective properties and is used in treatment of various liver diseases²⁵. Various studies indicate that Silymarin exhibits strong antioxidant activity²⁶ and shows protective effects against hepatic toxicity induced by a wide variety of agents by inhibiting lipid peroxidation²⁷. Higher total phenolic content has been known to contribute to the antioxidant activity of extracts²⁹, while antioxidant activity has also been linked to the hepatoprotective effect of some extracts²⁹. 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 extract³⁰. 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 rats³¹. The type of liver injury 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 damage³². 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 reinforcing 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 APAP³⁴. 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.

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