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HEPATOPROTECTIVE EFFECT OF LYCOPENE AGAINST PARACETAMOL-INDUCED HEPATIC DAMAGE IN ALBINO RATS

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ABSTRACT

Aim: The hepatoprotective effect of Lycopene was evaluated against paracetamol induced hepatic damage in albino rats. **Materials and Methods:** Liver function tests and biochemical parameters were estimated usingstandard kits. Livers were quickly removed and fixed in 10% formalin and subjected to histopathological studies. **Results:** There was a significant ($p < 0.05$) reduction in serum bilirubin levels with silymarin and lycopene 10mg/kg treated groups signifying protection against hepatic damage, lycopene 5mg/kg treated groups also showed significant change in bilirubin level. Similarly, significant ($p < 0.05$) reduction in the levels of serum transaminases were observed with all the treatment groups though more evident in the positive control and lycopene 10mg/kg treated groups. **Conclusion:** The results of this study strongly indicate that Lycopene may possess hepatoprotective action against paracetamol induced hepatic damage in rats.

KEYWORDS: Paracetamol, Lycopene, Silymarin, Heptoprotective.

INTRODUCTION

The liver is of vital importance in intermediary metabolism and in detoxification and elimination of toxic substances. The liver is often affected by a multitude of environmental pollutants and drugs, all of which place a burden on this vital organ and can damage and weaken it, eventually leading to diseases like hepatitis or cirrhosis.[1]

Paracetamol's hepatotoxicity is caused by its reactive metabolite. N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. Paracetamol

toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P450.[2] Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity.[3,4,5] In spite of tremendous strides in modern medicine, the treatment of liver disorders is inadequate and many formulations containing herbal extracts are used for regeneration of hepatic cells and for protection of the liver against damage.[6] Hepatic damage is associated with distortion of its metabolic functions and it is still a major health problem.[7]

Unfortunately many synthetic drugs used in the treatment of liver diseases are inadequate and also cause serious side effects.[8] In view of severe undesirable side effects of synthetic agents, there is growing interest in evaluating traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases. Therefore, an effective formulation using indigenous medicinal plants has to be developed with proper pharmacological experiments and clinical trials.[9]

The main objective of this study is to further understand the mechanism of lycopene's antioxidant action by evaluating the protective effect of orally administered lycopene pre-treatment on paracetamol induced rats. paracetamol is a hepatotoxic agent used to induce liver injury in experimental animals to check the efficiency of potential hepatoprotective agents.[10] The present study investigates the activity of the Lycopene against paracetamol-induced toxicity in comparison with silymarin a well-known antihepatotoxic agent.

MATERIALS AND METHODS

Experimental design

This experimental study was carried in models of paracetamol induced hepatotoxicity in albino rats. Lycopene was evaluated in paracetamol induced hepatic damage. The effects of lycopene was compared with silymarin, a proven hepatoprotective agent in this model of hepatotoxicity.

The study was conducted in strict accordance with the study protocol and CPCSEA guidelines Study animals were housed in the Central Animal House of our Institute, in an air-conditioned area with 12-15 filtered fresh air changes, temperature 22-30°C, relative humidity 30-70% six rats per cage were housed in polypropylene cages having husk paddy as the bedding, during the study. Twelve hourly light and dark cycles were maintained.

The model was standardized and hepatic damage was confirmed in the model. The effects of lycopene were evaluated in experimental models of paracetamol induced hepatic damage 30 albino rats of either sex weighing between 150-200 grams were used for the entire study. Lycopene was used in two doses of 5mg/kg and 10mg/kg based on the dose animal studies of lycopene in previous as hepatoprotective. Lycopene was administered orally. Daily, suspended in 0.5% CMC. Silymarin was administered in the dose of 50mg/kg, orally.

The effects of lycopene were evaluated in paracetamol induced hepatic damage, using silymarin as positive control. 24 Wistar rats were randomly allocated into four group's namely toxicant control (paracetamol 2gm/kg), silymarin (50mg/kg), lycopene (5mg/kg) and lycopene. (10mg/kg), each group containing 6 rats. The study drugs were administered for a duration of 7 days. On the 8th day, Induction of hepatic damage was carried out with paracetamol given orally in the single dose of 2g/kg. 24 hours following the administration of paracetamol, 2ml of blood was collected by puncturing the retro-orbital sinus and biochemical investigations was performed. Then the rats were euthanized by administering ketamine intraperitoneally. The liver was dissected out, washed in cold saline and blotted dry by placing it on tissue paper Weight and volume of liver was measured and processed further for histopathological examination.

Assessment of liver function parameters

At the end of the experimental period, animals were sacrificed by cervical decapitation under mild ketamine anesthesia, blood was collected and the serum was separated by centrifuging at 300 rpm for 10 min. The collected serum was used for the assay of marker enzymes. The serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel.[12] Alkaline phosphatase (ALP) was determined by the method of Kind and King.[13]

Assessment of biochemical parameters

The total bilirubin was estimated by Method of Malloy and Evelyn.[16] Immediately after sacrificing the animal, the liver was excised from the animals, washed in ice-cold saline, and the weight of the liver was recorded.

Histological studies

Livers were quickly removed and fixed in 10% formalin, dehydrated in gradual ethanol (50%–100%), cleared in xylene and embedded in paraffin. Sections (4–5 mm thick) were prepared and

then stained with hematoxylin and eosin dye for photo microscopic observations of the liver histologic architecture of the control and treated rats.

Statistical analysis

The results were expressed as mean ± standard deviation (S.D). Differences in liver function parameters and biochemical parameters were determined by factorial one-way ANOVA. Individual groups were compared using Tukey's test. Differences with $P<0.05$ were considered statistically significant.

RESULTS

There were no macroscopic changes observed in the liver any of the study groups. There was statically significant decrease in the liver weight and volume observed with grips that received silymarin 50mg/kg and lycopene 10mg/kg when compared with toxicant control. (Table 1)

Table 1: Effect of lycopene on liver Weight and Volume in rat model of paracetamol induced hepatotoxicity.

Groups	Liver weight (gm/100gm body weight)	Liver volume (ml/100gm body weight)
Normal control	4.82±0.11	5.62±0.24
Toxical control	5.81±0.12	9.62±0.17
Standard (Silymarin 50mg/kg)	3.81±0.12	5.64±0.17
Test Ⅰ (Lycopene 5mg/kg)	4.62±0.43	5.13±0.22
Test Ⅱ (Lycopene (10mg/kg)	3.95±0.41	5.90±0.18

All values represent Mean ±SD (n=6).

Biochemical parameters

There was a significant ($p <0.05$) reduction in serum bilirubin levels with silymarin and both doses of lycopene 5mg /kg and 10mg/kg treated groups signifying protection against hepatic damage. Similarly significant ($p <0.05$) reduction in the levels of serum transaminases were observed with positive control and lycopene 5 and 10mg /kg treated groups. (Table 2)

Table 2: Effect of lycopene on biochemical parameters in rat model of paracetamol induced hepatotoxicity.

Groups	Serum bilirubin (mg/dl)	Aspartate transaminase (IU/ml)	Alanine transaminase (IU/ml)	Alkaline phosphatase(IU/ml)
Normal control	0.53±0.03	81.10±8.52	75.43±8.33	90.96±6.66
Toxicant control	0.93±0.08	308.40±17.10	149.05±11.43	248.01±22.23
Standard (Silymarin 50mg/kg)	0.28±0.04	140.02±12.35	62.53±7.10	126.02±10.40
Test Ⅰ (Lycopene 5mg/kg)	0.48±0.03	198.26±12.16	68.32±05.28	180.03±15.33
Test Ⅱ (Lycopene 10mg/kg)	0.36±0.06	137.24±13.39	66.42±06.32	122.01±11.30

All values represent Mean ±SD (n=6) $p<0.05$ using one way ANOVA with post hoc Tukey's test (versus toxicalnt control)

Table 3: Histopathological changes.

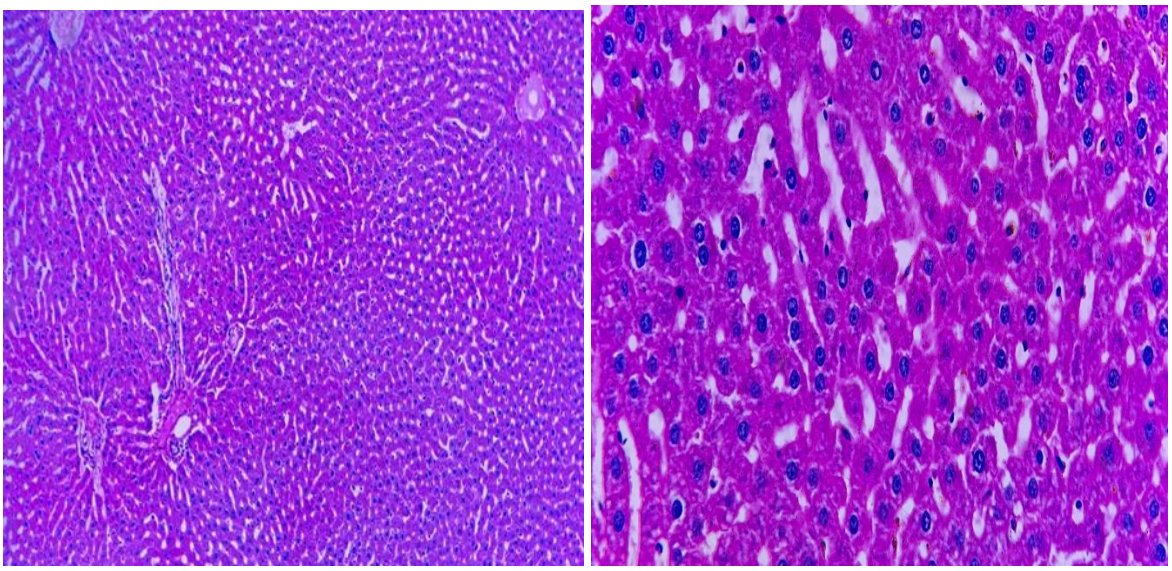
Groups	No of animals showing						
	Degeneration				Necrosis		
	0	Ⅰ	Ⅱ	Ⅲ	0	Ⅰ	Ⅱ
Normal control	0	4	2	0	1	5	0
Standard (Silymarin 50mg/kg)	1	5	0	0	4	2	0
Test Ⅰ (Lycopene 5mg/kg)	0	4	2	0	1	5	0
Test Ⅱ (Lycopene 10mg/kg)	1	4	1	0	4	2	0

Out of the six animals in the normal group four showed grade I degeneration while the remaining, two showed grade II degeneration. Five animals showed presence of 1-2 necrotic cells per high power field (Grade 1) while one animal showed no necrosis. Similar changes were seen in the group that received lycopene in the dose of 5mg/kg with regard to the number of animals.

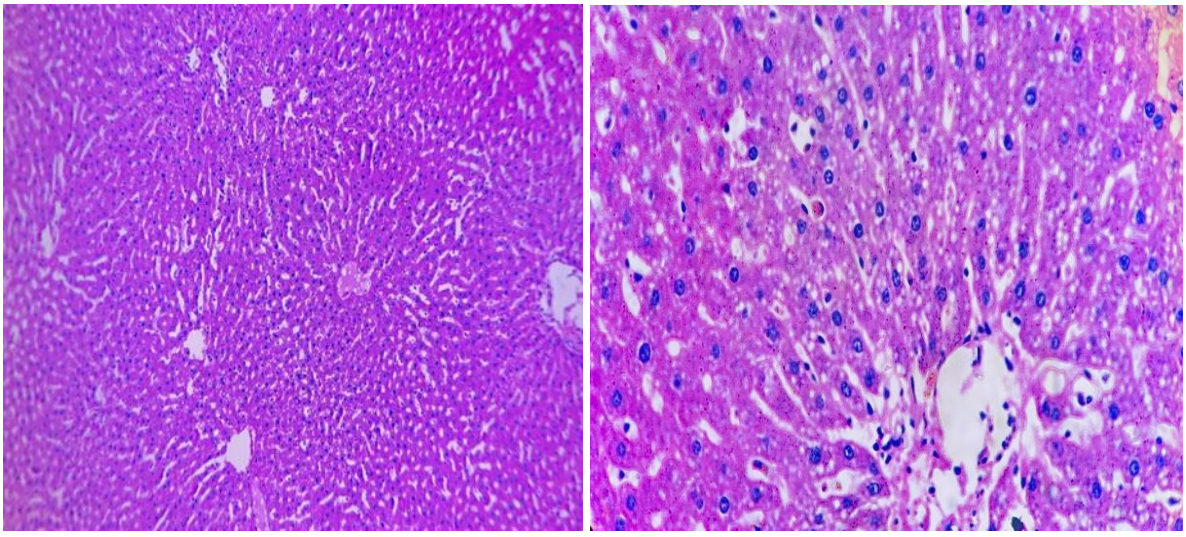
In the silymarin treated group, 4 animals showed no necrosis while two animal showed necrosis and Minimal degenerative changes were seen in 5 animals.

In lycopene 10mg/kg treated group, one animal showed no degeneration and grade II degeneration each, with remaining 4 animals showing grade I degeneration. Two animal in the lycopene 10mg/kg treated group showed necrosis while 4 showed near normal hepatic

parenchyma. (Table 3, Figure 1).



1. Normal control **2. Standard (Silymarin 50mg/kg)**



3. Test Ⅰ (Lycopene 5mg/kg) **4. Test Ⅱ (Lycopene 10mg/kg)**

Figure 1: (1 to 4) effects of study drugs on histopathology in rat model of paracetamol induced hepatic damage.

DISCUSSION

While standardizing this model, Serum bilirubin, AST, ALT and ALP as biochemical parameters were chosen. Morphological parameters such as liver weight and liver volume were measured to find changes in liver morphology. Structural alterations the liver due to ongoing insults was confirmed by doing histopathological examination of liver at the end of study.

During standardization of our study, none of the study animals died during the study duration. Paracetamol produced significant ($p < 0.05$) elevation in serum bilirubin AST and ALT levels, compared to the toxicant control. Lycopene has caught the attention of investigators as a potential hepatoprotective due to its antioxidant, anti-inflammatory and anti-proliferative properties.[30]

Liver is the chief target organ of lycopene accumulation in the body. After oral administration, lycopene is rapidly absorbed and gets accumulated in the liver, with a lesser amount going to the spleen. Safety of lycopene has been proved beyond doubt in multiple toxicity studies. No significant toxic effects were observed with lycopene up to 2000mg/kg body weight when administered orally. Animals were observed for 24 hours.

There is a need of evaluation of lycopene in other models of hepatotoxicity with higher doses given its wide safety margin. Further studies are essential to expatiate its mechanism of action. In future, lycopene would be a potential hepatoprotective agent against drug induced hepatotoxicity in clinical use. Especially in the prevention/treatment of paracetamol induced hepatotoxicity.[32,33]

CONCLUSION

It is concluded that lycopene emerge hepatoprotective effect against paracetamol induced hepatic damage in rats. Lycopene needs to be evaluated in other models of hepatotoxicity and further studies are required to delineate its mechanism of action. Lycopene might be a potential hepatoprotective for clinical use in future.

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