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Investigation of hepatoprotective effects of piperine and silymarin on D-galactosamine induced hepatotoxicity in rats

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of combination of phytoconstituents of piperine and silymarin against D-galactosamine induced liver damage in albino rats. The phytoconstituents piperine (100 mg/kg) was administered orally to the animals with hepatotoxicity induced D-galactosamine (400 mg/kg for two days). Silymarin (50 mg/kg) was given as standard hepatoprotective drug. All the test drugs were administered orally in the suspension form to the animals. The combination of silymarin and piperine were also given to animals to compare with individual groups as well as toxicant group. Combination of piperine and silymarin were effective in protecting the liver against the injury induced by D-galactosamine in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin. These biochemical observations were also supplemented by histopathological observations of the liver sections. It was concluded from the result that the combination of piperine and silymarin reduces the hepatoprotective activity against D-galactosamine induced hepatotoxicity in rats.

Key words: Piperine, D-galactosamine, silymarin, hepatoprotection

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INTRODUCTION

The liver is a very important organ which regulates various physiological processes in the body. It can detoxicate toxic substances and make useful ones. Therefore the damage which is caused by hepatotoxic agents is of crucial effect to the body as it prevents the liver of its principal functions [1]. Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and reduction in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin levels are elevated [2]. Conventional or synthetic drugs used in the treatment of liver diseases are unsatisfactory and sometimes can have serious side effects. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of hepatic disorders. In view of severe undesirable side effects of synthetic agents, there is increasing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to have hepatoprotective activity [3].

Liver injuries induced by D-galactosamine are the best characterized system of xenobiotics induced hepatotoxicity and commonly used models for the screening of anti – hepatotoxic and/or hepatoprotective activities of drugs. Hence D-galactosamine mediated hepatotoxicity was chosen as the experimental model [4].

Piperine is the alkaloids responsible for the pungency of Piper species which is obtaining from unripe fruits belonging to family Piperaceae, widely cultivated in India, Ceylon and other tropical countries. It is a piperidine derivative with multiple pharmacological and therapeutic activities. The traditional use includes hepatoprotective, CNS depressant, antipyretic, anti-inflammatory, antibacterial and anti-tumor activities [5]. In human's, consumption of 20 mg of pure piperine resulted in no adverse reactions. Hence, this dose was considered as a safer dose to evaluate the therapeutic efficacy of piperine [6].

Piperine is principle ingredient in pepper. Several uses of pepper were described since ancient times in Ayurvedic and Chinese herbs literature. However its use for hepatoprotective activity was never reported. For the first time, the hepatoprotective activity of piperine was reported by Indu Bala Koul and Aruna Kapil, from Regional Research Laboratory, Jammu, India. Since then several direct or indirect evidence has been routinely reported regarding its hepatoprotectivity. Thus present study was conducted to evaluate the hepatoprotective activity of the piperine by using D-galactosamine induced hepatic injury in albino rats.

MATERIALS AND METHODS

Drug and chemicals

Silymarin was obtained from NIPER, Punjab. D-galactosamine was purchased from Venus Chemical Centre, Surat, India. The solvent and chemicals used were of analytical grade. Piperine was extracted from Piper nigrum.

Plant material collection

The fresh plants were collected during the month of July of 2010 from campus of Shree Dhanvantari Pharmaceutical and Research Institute, Surat. The plant was identified by a botanist of the botany department of SDPARC, Surat. After authentification, the plant fruits were cleaned and shaded dried at room temperature and milled into coarse powder by a mechanical grinder.

Preparation of plant extracts

20 g of fruit powdered was extracted with 250 ml ethanol (95 %) in soxhlet apparatus for 3 h. The solution was filtered and kept under vacuum on a water bath at 60˚C. 20 ml of 10% alcoholic KOH was added with constant stirring to the concentrate. Then the extract was filtered and allowed the alcoholic solution to stand overnight where up on needles of piperine separated out. Separated piperine was collected and kept for drying.

Animals

Albino rats (wistar) weighing 150 – 200 g of either sex were procured from Shri Dhanvantari Pharmaceutical and research institute, Kim, Surat. The animals were acclimatized for one week under laboratory conditions. They were housed in cages and maintained at 27˚C ± 2˚C and relative humidity of 30 – 70 % with a 12 h dark / light cycle. They were fed with standard rat feed and water was provided. The litter in cages was renewed thrice a week to ensure hygiene and maximum comfort for animals.

Administration of doses

The test substances were administered in a single dose by oral route. Animals were found prior to dosing, following period fasting, the animals were weighed and test substance was administered. After dosing, food was withheld for a further 3-4 h in rats. D-galactosamine was induced by i.p. route to animals.

D-galactosamine induced hepatotoxicity

The animals were divided into five groups, each group with five animals for recording enzymatic levels and histopathology during the evaluation. Group I served as normal control and received normal saline water (1 ml/ kg p.o.) daily for 10 days. Group II received D-galactosamine (400 mg/kg) by i.p route) on last two days. Group III received silymarin (50 mg/kg p.o.) for 10 days and D-galactosamine (400 mg/kg by i.p route) on last two days. Group IV received piperine (100 mg/kg p.o.) for 10 days and D-galactosamine (400 mg/kg by i.p route) on last two days. Group V received combination of piperine (100 mg/kg p.o.) and Silymarin (50 mg/kg p.o.) for 10 days and D-galactosamine (400 mg/kg by i.p route) on last two days.

Biochemical parameter estimation

All the rats in all the groups were sacrificed on 11th day under light anaesthetic ether. Blood from each rat was collected through retro orbital under ether anaesthesia for biochemical investigation i.e. SGOT, SGPT, ALP and total bilirubin estimation. Blood was allowed to coagulate at 37^{0}C for 30 min and the serum was separated by centrifugation at 2500 rpm for 10 min.

Histopathological investigation

The liver from each animal was removed after dissection. The liver lobes were fixed for 48 h in 10% formalin and were embedded in paraffin. Subsequently, 5 μ sections of livers were stained with haematoxylin and eosin. These sections were observed under light microscope for histological changes and compound to normal liver physiology.

Statistical analysis

The results of biochemical estimation were expressed as mean ± standard error of mean (n = 5) for determination of significant inter group difference was analyzed separately and one-way analysis of variance (ANOVA) was carried out by using Prismpad software 5.02 version. Dunnet's test was used to compare group I with Group II, Group III, Group IV and Group V to find the significant changes of the individual groups.

RESULTS AND DISCUSSION

The effects of phytoconstituents on rats induced by D-galactosamine hepatotoxicity were observed in serum enzymes levels like SGPT, SGOT, ALP and total bilirubin. A significant change in the levels of SGPT, SGOT, ALP and total bilirubin was observed. The in vitro hepatoprotective activity of phytoconstituents against D-galactosamine induced toxicity was potent and comparable with that of standard silymarin. Hence in vivo hepatoprotective activity using D- galactosamine intoxicated rats. Intoxication of rats with D-galactosamine (400 mg/kg) significantly altered the biochemical parameters when compared with the normal control rats. A significant increased in the levels of SGPT, SGOT, ALP and total bilirubin in toxicant group while decreased in other groups.

Control group showed serum values of ALP, SGPT, SGOT and total bilirubin in rats were found to be 375.6 ± 1.72, 53.60 ± 1.21, 175.2 ± 1.49 and 0.23 ± 0.009 IU/L (n=5), respectively, while a toxic dose of D-galactosamine (400 mg/kg) significantly raised ($p < 0.05$), the respective serum enzymes values to 446.6 ± 1.44, 110.0 ± 1.14, 238.2 ±0.66 and 0.54 ± 0.008 IU/L, respectively. Group III was treated with standard silymarin, shows the respective serum enzymes values to 423.6 ± 1.72, 69.40 ± 1.47, 196.2 ± 0.80 and 0.49 ± 0.005 reduced as compared to D-galactosamine induced animals respectively. The serum enzymes values in piperine treated animals were found to be 410.4 ± 0.93, 62.40 ± 1.47 , 184.2 ± 0.86 and 0.44 ±

0.009 which were found decreased as compared to standard silymarin treatment. When the combination of piperine and silymarin were shown most significant reduction in serum enzymes values found to be 395.2 ± 1.39, 64.40 ± 0.81, 187.2 ± 1.02 and 0.35 ± 0.01, respectively, which shows decrease in serum enzymes values when compared with silymarin and piperine. ANOVA indicates significant differences among the groups. Piperine reduced the elevated the reduced levels of SGPT, SGOT, ALP and total bilirubin [Table 1].

Table 1: Measurement of biochemical parameters

Groups	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total bilirubin (IU/L)
Group I	Normal saline	53.60 ± 1.21	175.2 ± 1.49	375.6 ± 1.72	0.23 ± 0.009
Group II	D-galactosamine control	110.0 ± 1.14[a]	238.2 ± 0.66[a]	446.6 ± 1.400[a]	0.54 ± 0.008[a]
Group III	Standard silymarin	69.40 ± 1.470[a]	196.2 ± 0.80[a]	423.6 ± 1.72[a]	0.49 ± 0.005[b]
Group IV	Piperine extract	62.40 ± 1.47[a]	184.2 ± 0.86[a]	410.4 ± 0.93[a]	0.44 ± 0.009[b]
Group V	Tablet prepared by using silymarin and piperine	64.40 ± 0.81[a]	187.2 ± 1.02[a]	395.2 ± 1.39[a]	0.35 ± 0.01[b]

Values are the mean ± SEM, n = 5, $p \leq 0.001$[a] compared to D-galactosamine group, $p < 0.05$ [b] compared to control group, Serum enzymes levels after 11th day

The hepatoprotective effect was confirmed by histological examination of the liver tissue of control and treated animals. Histological profile of the control animals showed normal hepatic architecture with distinct hepatic cells well preserved cytoplasm sinusoidal spaces and central vein [Figure 5A]. The histological architecture of D-galactosamine treated liver section showed massive fatty changes, ballooning degeneration and the loss of cellular boundaries [Figure 5B]. However necrosis was not observed in any groups which indicate that sufficient hepatotoxicity does not seems to have developed in the animals so as to cause the necrosis of liver. D-galactosamine treated liver sections showed ballooning degeneration, the loss of cellular boundaries, nuclear pycnosis and karyolysis. However administration of extracts at higher doses of D-galactosamine (400 mg/kg) significantly normalized these defects in the histological architecture of the liver. The phytoconstituents like piperine showed excellent protection to liver architecture almost to the level of the Silymarin treated groups, showing its potent hepatoprotective effects in animal model [Figure 5C].

D-galactosamine acts directly or indirectly alters some status and makes certain more susceptible or oxidative stress. D-galactosamine is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis. D-galactosamine is also responsible to loss in the activity of pumps and for cell death**.** D-galactosamine intoxication is known to cause marked elevation in liver enzyme levels. Silymarin is used as standard hepatoprotective compound since it is reported to have a protective effect on the plasma membrane of hepatocytes [Figure 5D]**.** D-galactosamine

induced extensive liver damage within a period of 24 h following intra peritoneal administration. As a result of this, the phytconstituents were administered to rat when induced by D-galactosamine, decrease the serum enzyme levels and normalized the liver as compared to D-galactosamine. Accumulation of fat in liver and necrosis in centrilobular region of the liver occurs.

In the present study, the combination of piperine and silymarin show the good significant effect as compared to other groups [Figure 5E]. They are very effective in reducing the injurious effect which was damaged by D-galactosamine. From histopathological study, it also reveals that the combination of piperine and silymarin normalized the damage of liver as compared to toxicant group. This shows that the combination of piperine and silymarin show good effect in animals when induced by D-galactosamine hepatotoxicity. So it supports the results of piperine and silymarin combination as compared to other groups.

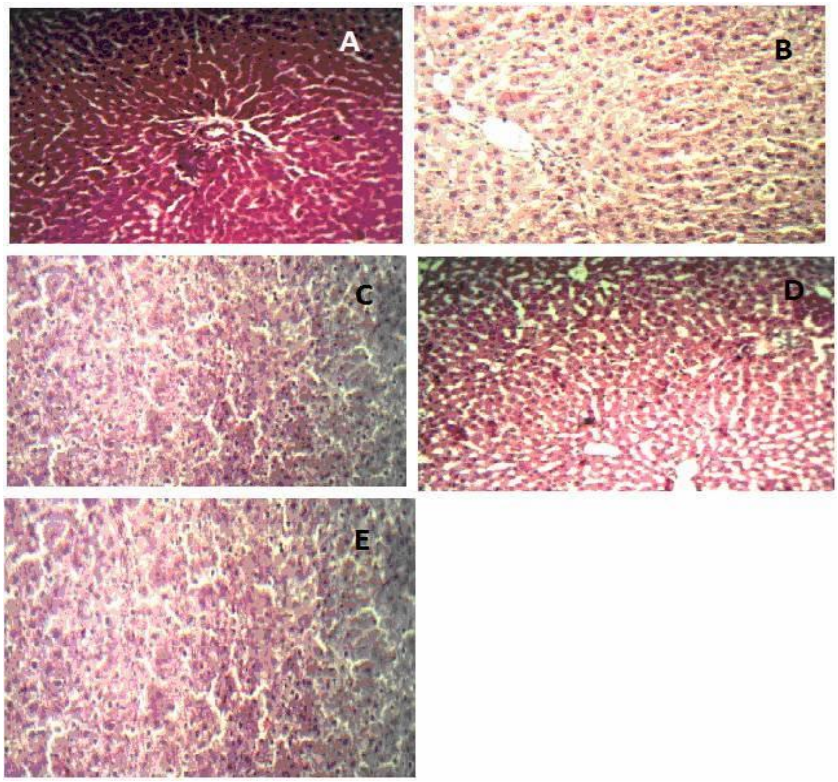

Figure 5: Histology of the liver of various vehicles treated rats. (A) Group I, (B) Group II, (C) Group III, (D) Group IV and (E) Group V

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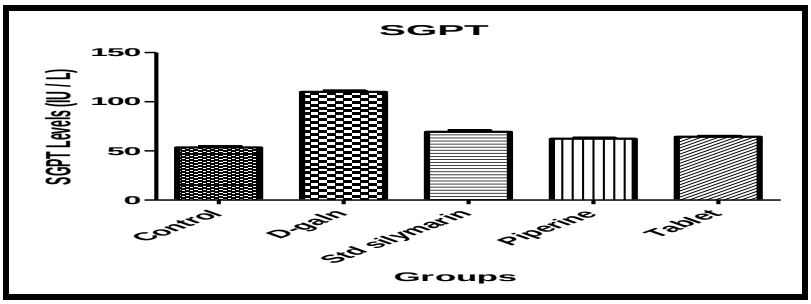

Figure 1 Comparison of SGPT enzymes levels in different groups induced by D-galactosamine hepatotoxicity

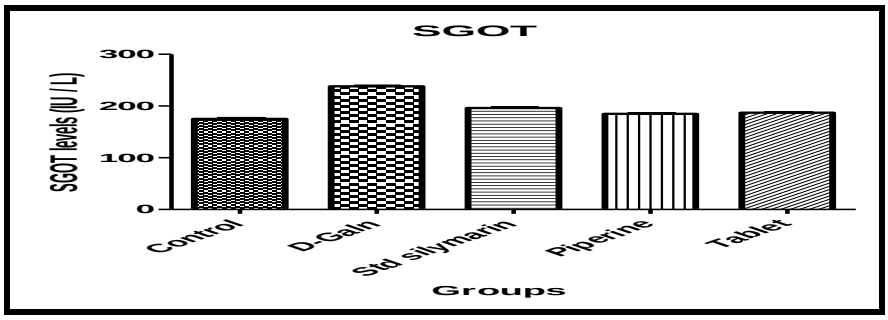

Figure 2: Comparison of SGOT enzymes levels in different groups induced by D-galactosamine hepatotoxicity

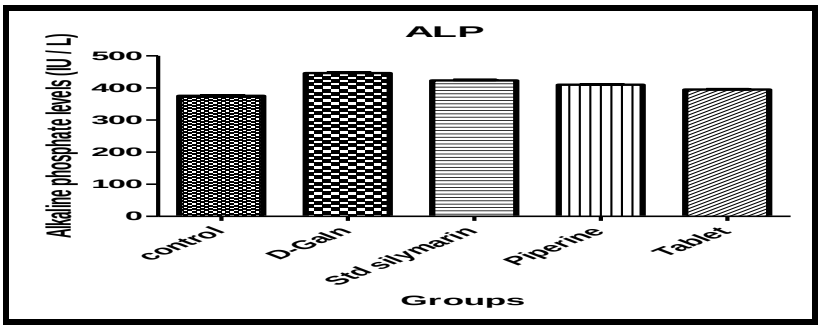

Figure 3: Comparison of ALP enzymes levels in different groups induced by D-galactosamine hepatotoxicity

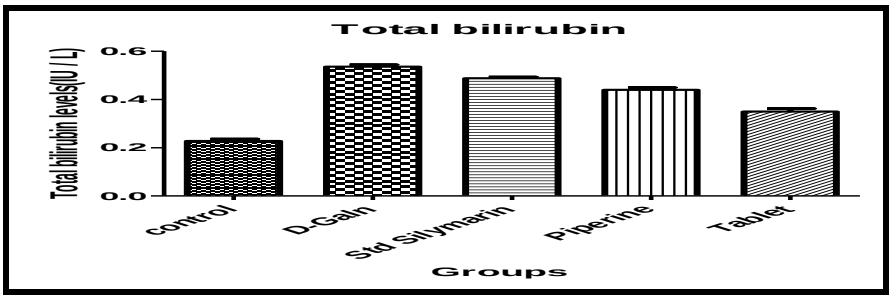

Figure 4: Comparison of Total bilirubin enzymes levels in different groups induced by D-galactosamine hepatotoxicity

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CONCLUSION

From the present study, it reveals that combination of piperine and silymarin shows a good significant effect as compared to individual phytoconstituents was given to the animals. When combination of piperine and silymarin were given to the animals then they showed protectant effects on liver as compared to toxicant group and other individual group. So the piperine with silymarin enhances the activity and reduces the damage of liver. From histopathological study, it also reveals that combination normalized the livers.

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