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Hepatoprotective activity of silymarin against paracetamol induced liver toxicity in albino rats

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Abstract
Paracetamol overdose is a common cause of liver injury. Silymarin is a hepatoprotective agent widely used for the treatment of liver injuries of different origins. In order to evaluate the possible beneficial effects of silymarin, albino rats were pre-treated with silymarin (100 mg/kg b.wt. *per os*) once daily for seven days. On the 8th day of the experiment, in 24 hour fasted rats, paracetamol was given at the dose rate of 2 g/kg body weight. Paracetamol produced a significant effect on the liver, which was evidenced by an increased level of AST and ALT. The adverse effect of paracetamol was also observed in the kidney, as evidenced by an increased level of BUN and serum creatinine. Changes in haematological parameters were also observed. Silymarin-treated rats exhibited a significant reduction in paracetamol-induced liver injury, assessed according to AST and ALT examination, and also showed a positive effect on BUN, serum creatinine, and haematological parameters as well.

Keywords: Silymarin, paracetamol, AST and ALT, BUN and serum creatinine

Introduction
It is unambiguous that human life is impossible without nature. Humans require three essential necessities: food, clothing, and shelter, and now the fourth one is good health, which is provided by the plant kingdom. The plant kingdom represents a rich house of organic compounds, many of which have been used for medicinal purposes and could serve as a lead for the development of novel agents with good efficacy in various pathological disorders. (More *et al*., 2012) [22]. Herbal drugs are the main constituents of traditional medicine and are a common ingredient in Ayurveda, the oldest known medical system (DB *et al*., 2018) [9], which is widely used in India, Sri Lanka, and many other nations (Shrikumar *et al*., 2007) [30].
Herbal medicine has a very long history and possibly existed before modern Homo sapiens. People in past civilizations systematically and scientifically gathered knowledge about herbs and created evident herbal pharmacopoeias. The earliest written accounts of these initiatives can be found in texts from around 5000 years ago in Indian, Chinese, Egyptian, Greek, Roman, and Syrian records (Inamdar *et al.*, 2008) [16].
Silymarin is an active constituent of the plant *Silybium marianum* (milk thistle, family Asteraceae) (Pradhan *et al*., 2006) [25] is one of the oldest plants of ancient times used in the treatment of liver and gall bladder disorder, including jaundice, cirrhosis and hepatitis. It possesses diverse pharmacological activities, including hepatoprotective (Mahli *et al*., 2015 and Cacciapuoti *et al*., 2013) [21, 6] antioxidant (Anthony and Saleh, 2017) [2] and anticancer, cardio protective etc. The commercial silymarin preparations contain several different flavonoids, like silibinin (silybin A and B), silichristin, silidianin and isosilibinin (isosilybin A and B). Silymarin has membrane-stabilizing property which promotes hepatocyte regeneration, reduces inflammatory reaction and inhibits fibrogenesis (Brantley *et al*., 2010) [5].
Silymarin shows hepatoprotective activity against partial hepatectomy models and toxic models in experimental animals by using acetaminophen, carbon tetrachloride ethanol, D-galactosamine (Dixit *et al*., 2007) [10]. The signs of lipid peroxidation (malondialdehyde content) and the parameters of antioxidant capacity (activities of superoxide dismutase, catalase and glutathione peroxidase) beneficially changed after silymarin treatment. The pharmacokinetic studies of silymarin have revealed poor absorption, rapid metabolism and ultimately poor oral bioavailability of silymarin (Wen *et al*., 2008) [34].
Acetaminophen (N-acetyl-para-aminophenol, paracetamol, APAP) inhibit cyclooxygenase enzyme (COX) and prostaglandin synthesis in the brain and central nervous system (CNS)

respectively. A single dose of Paracetamol can induce clinically relevant liver injury and has become a standard model for hepatotoxicity in the pharmacology research. (Chakrabarti *et al*., 1978) [7].

In the liver, paracetamol is metabolised via one of three pathways: glucuronidation, sulfation, or the hepatic cytochrome P450 enzyme system (Ghaffar *et al*., 2014) [11] conjugation events such as glucuronidation and sulphonation, these conjugates convert into nontoxic compounds which are then excreted with the urine. Only a very small portion is excreted unchanged in the urine (Ullah *et al*., 2022) [32]. A small amount of paracetamol was metabolised by the cytochrome P450 enzyme, mainly CYP 2E1 (Larson *et al*., 2005) [20], resulting into highly reactive and toxic metabolite, N-acetyl-benzoquinone imine (NAPQI), which was then inactivated by glutathione (Lancaster *et al*., 2015) [19]. At toxic dosages, however, the cytochrome P450 system and glutathione become saturated, resulting in NAPQI accumulation and subsequent interaction with the cellular protein resulting in cellular damage, liver necrosis, jaundice, diminished synthetic capability, increased bilirubin accumulation, and fulminant hepatic dysfunction (Ibrahim *et al*., 2013) [15].

Materials and Methods

This study was undertaken to determine the hepatoprotective potential of silymarin against paracetamol-induced liver damage in albino rats. The study was conducted in healthy adult inbred albino rats of either sex weighing 150-200 g. The rats were kept in polycarbonate cages and maintained under hygienic conditions in Lab Animal House at the College of Veterinary Science and Animal Husbandry, Jabalpur. The rats were given a standard pellet diet and free access to drinking water. Animals were kept under observation for two weeks prior to the commencement of the experiment. During this period, animals were subjected to clinical examination in order to exclude any possibility of the disease condition. All necessary management procedures were adopted to keep the animals free from undue stress.

The experimental protocol and use of animals in the experiment was approved by Institutional Animal Ethics Committee (IAEC) No: 116/IAEC/Vety/2018. Guidelines of CPCSEA were followed for the care and management of animals during the entire period of experimentation.

Drugs and chemicals

Paracetamol and silymarin were purchased from Micro Labs Ltd., Bengaluru, Karnataka and other essential chemicals used in this study were purchased from Hi Media Laboratory Pvt. Ltd., Mumbai.

Animals' treatment

Experimental rats were randomly divided into three groups having four animals in each group and were kept in separate cages. The first group served as normal control and was only given normal saline orally for 10 days. To the second group, on the 8th day of the experiment, a single dose of paracetamol @ 2 g/kg bw was given orally for the induction of liver damage. To the 3rd group of rats, Silymarin was given @ 100 mg/kg bw, orally for 7 consecutive days, and on the 8th day single dose of paracetamol was given @ 2 g/kg bw, orally.

Blood collection

All the rats were sacrificed on the 10th day of study by decapitation method. After decapitation, blood was collected in EDTA and non-EDTA coated test tubes respectively for evaluation of haematological and biochemical parameters.

The blood samples collected in sterile EDTA coated test tubes were used for the evaluation of haematological parameter. The blood collected in Non EDTA test tubes were allowed to clot for 45 minutes at room temperature and serum was separated by centrifugation at 4000 rpm for 20 min. The semi-automatic biochemical analyser was used to evaluate the level of serum aminotransferases such as AST (IU/L) and ALT (IU/L) and alkaline phosphatase (ALP) (IU/L), albumin (g/dl), globulin (g/dl), total protein (g/dl), creatinine (mg/dl), and BUN (mg/dl).

Result and Discussion

Paracetamol in normal doses, primarily metabolises by conjugation with sulfate and glucuronic acid. In the metabolism of paracetamol highly reactive metabolite N-acetyl-p- benzoquinone imine (NAPQI) is formed. In condition of overdose, the generation of NAPQI exceeds the capacity of detoxification by reduced glutathione (GSH) this causes liver toxicity by oxidative damage (Aubert *et al*., 2012) [3].

In our study, the mean value of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the paracetamol treated group was significantly higher than control group. Handa and Sharma (1990) [14] observed the elevated levels of liver biochemical marker enzyme including ALT in paracetamol induces toxic liver model. Setty *et al*. (2007) [29] noticed the markedly increases in ALT value by paracetamol administration at the dose rate of 2 g/kg b. wt. in rats.

The mean value of AST and ALT in the silymarin and paracetamol treated group III was the lowest among all the treatment groups. The lower values of AST and ALT in the silymarin and paracetamol treated groups indicated silymarin produced hepato-protective effect. Vetriselvan and Subasini (2012) [33] observed reduction in AST value in silymarin (50 mg/kg bw, oral) treated animals in paracetamol induced liver damage model. Further, Kolakota *et al*. (2017) [18] in his study also observed marked decrease in aspartate aminotransferase (AST) value, after silymarin treatment, in paracetamol induced liver damage model in rats.

The mean value of ALT in silymarin treated group was lowest among all the treatment groups. The result suggests that the silymarin provided protection against paracetamol induce liver damage. Similarly, Muriel *et al*. (1992) [23] also showed the protective effects of silymarin and demonstrated significantly decreases in ALT level in paracetamol-induced lipid peroxidation and glutathione depletion. Nagalekshmi *et al*. (2011) [24] analysed the hepatoprotective potential of silymarin against paracetamol induced hepatotoxicity and observed significant decrease in ALT value in silymarin treated animals (rats) in comparison to hepatotoxic Swiss albino mice.

The liver is the major source for most of the serum proteins, the parenchymal cells of liver are responsible for the synthesis of albumin, fibrinogen and other coagulation factors and most of α and β globulins. Reducing the concentration of albumin mainly occurs due to increased vascular permeability during acute inflammation and its release into intercellular spaces. A low serum albumin indicates poor liver function and so the reduction in albumin level is generally suggestive of liver disease. Albumin binds to drugs or chemicals and facilitates their transportation (Thapa and Walia, 2007) [31].

Paracetamol in this research caused decrease in total protein (TP), albumin and globulin in serum. A noticeable decrease in total protein may be the result of reducing the number of cells responsible for protein synthesis in the liver due to necrosis (Goldwasser and Feldman, 1997) [12].

The mean values of albumin, globulin, and total protein which are given in Table 01 were higher in the control group than in the paracetamol-treated group. The mean values of albumin, globulin, and total protein are slightly higher in the group that was treated with silymarin. The higher values of albumin, globulin, and total protein in the silymarin treated groups III indicate that silymarin produces hepatoprotective activity.

Paracetamol toxicity produces acute tubular necrosis, which is one of the main causes of acute renal failure (Blantz, 1996) [4]. Serum urea and creatinine levels may be indicators of acute tubular necrosis induced by paracetamol (Cobden *et al*., 1982 and Blantz, 1996) [8, 4]. For evaluation of the effect of silymarin on kidney, creatinine and BUN were evaluated in different treatment groups.

The mean serum creatinine in paracetamol treatment group was significantly higher than in the control group. Simiarly, Isik *et al*. (2006) [17] also observed significantly increased creatinine levels in paracetamol treated group in comparison to control group of rats. Yousef *et al*. (2010) [35] also found significantly (p<0.05) higher levels of plasma creatinine in paracetamol treated animals than normal control group.

In the silymarin treated group III, serum creatinine was lower than in the groups that was treated only with paracetamol (group II). The lower serum creatine levels in group III indicated that silymarin has a reno-protective effect (Table-01). In their study, Gopi *et al*. (2010) [13] also showed the protective effects of silymarin and demonstrated significant decrease in serum creatinine level in paracetamol-induced nephrotoxicity. Ramachandran *et al*. (2012) [27] also observed marked decrease in serum creatinine, after treatment with silymarin, in paracetamol induced kidney damage in rats.

BUN is the most frequently used clinical indices for estimating renal function. Non protein nitrogenous substances such as BUN are increased when renal function is below 30% of its original capacity. The mean value of serum BUN in the paracetamol treatment group was significantly higher than in the control group. In the silymarin and paracetamol treated groups, serum BUN is lower than in the groups that are treated with paracetamol along (group II). The lower serum BUN levels in group III indicated that silymarin has a reno protective effect (Table-01). Ramachandran *et al*. (2012) [27] also observed marked decrease in serum BUN value, after treatment with silymarin, in paracetamol induced kidney damage in rats.

Paracetamol toxicity damaged the hepatic parenchyma, affecting erythropoiesis and also associated with decreased erythrocytes in the circulation resulting in reduced concentration of Hb. Additionally, the disintegration of erythrocytes in the circulation might also result in the reduction of haemoglobin concentration. Likewise, Yousef *et al*. (2010) [35] also reported that paracetamol caused a significant decrease in Hb concentration.AL-Harbi *et al*. (2015) [1] demonstrated that on administration of paracetamol for 30 successive days a significant decrease (P<0.05) in Hb level was observed.

As shown in Table 02, the mean observed value of Hb in the paracetamol alone administered group II significantly decreased as compared to the control group. The mean value of Hb in the silymarin treated group III was significantly greater than in the paracetamol treated group.

The RBC value in the control group was significantly higher in comparison to the paracetamol-treated group II. Moreover, the RBC value in the paracetamol treated group II was the lowest among all the study groups. The mean value of RBC in the silymarin treated group III significantly higher than in the paracetamol treated group II (Table-02). The mean value of WBC in paracetamol treated group is significantly higher in comparison to the control group. In the silymarin treatment group II the mean value of WBC significantly lower in comparison to the paracetamol alone treated group.

Samuel *et al*. (2015) [28] observed a significant decline in RBCs, Hb and PCV, with a significant increase in WBC count in rats given paracetamol (300 mg/kg b. wt. intraperitoneal for 2 days). A substantial increase in total leukocytic count could be due to the body's defensive mechanism attempting to protect the body against infection following liver damage.

Table 1: Effect of silymarin against paracetamol induced toxicity on different biochemical parameter

Group	Treatment	AST (IU/L)	ALT (IU/L)	Albumin (g/dl)	Globulin (g/dl)	Total Protein (g/dl)	Creatinine (mg/dl)	Bun (mg/dl)
I	Normal saline control	76.51^{d}±1.57	86.00^{d}±2.55	3.10^{a}±0.08	4.91^{a}±0.15	8.03^{a}±0.18	0.71^{b}±0.02	11.78^{b}±0.26
II	Paracetamol control (8^{th} day)	116.45^{a}±3.26	139.43^{a}±3.56	2.55^{c}±0.03	4.21^{c}±0.04	6.86^{c}±0.13	0.84^{a}±0.03	14.10^{a}±0.23
III	Silymarin (1-7 day) + PCM (8^{th} day)	76.26^{d}±1.49	84.67^{d}±1.81	2.86^{b}±0.07	4.59^{b}±0.07	7.46^{b}±0.07	0.75^{ab}±0.02	12.00^{b}±0.28

Table 2: Effect of silymarin against paracetamol induced toxicity on different haematological parameters

Group	Treatment	Hb (gm/dl)	RBC ($x10^6$ /cu.mm)	WBC ($x10^3$ /cu.mm)	N (%)	L (%)	M (%)	E (%)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
I	Normal saline control	14.65^{a}±0.33	8.34^{a}±0.16	4.30^{c}±0.31	26.55^{b}±0.88	74.16^{a}±0.54	1.83±0.30	1.66±0.42	46.07±1.20	37.32^{a}±1.16	17.74±0.26	32.63±0.37
II	Paracetamol control (8^{th} day)	13.40^{b}±0.38	7.49^{b}±0.14	6.35^{a}±0.33	31.50^{a}±0.92	68.71^{b}±0.85	1.33±0.21	2.00±0.36	42.96±1.37	32.98^{b}±0.67	18.28±0.32	31.76±0.49
III	Silymarin (1-7 day) +PCM (8^{th} day)	14.52^{a}±0.15	8.12^{a}±0.13	5.40^{b}±0.21	24.33^{c}±0.66	73.83^{a}±1.30	1.33±0.21	1.33±0.21	45.46±0.53	37.70^{a}±0.81	17.88±0.24	31.94±0.29

N=Neutrophil, L=Lymphocyte, M=Monocyte, E=Eosinophil, PCV= packed cell volume, MCV= Mean corpuscular volume, MCH=Mean Corpuscular Haemoglobin, MCHC= Mean corpuscular haemoglobin concentration

Conclusion

Based on the information gathered from the present study, it can be concluded that, oral administration of higher dose of paracetamol causes significant hepatotoxicity as evident by alteration in haematological and biochemical parameters in rats. Silymarin, on oral administration, protected the liver from paracetamol induced hepatotoxicity and causes improvement of biochemical and haematological parameters in albino rats.

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