EFFECT OF *SILYBUM MARIANUM* SEED EXTRACTS ON BIOCHEMICAL PROFILE IN RABBIT LIVER

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ABSTRACT

In the present study the hepatogenic effect of *S. marianum* seeds was evaluated against paracetamol induced hepatotoxicity in rabbit. Aqueous extract (AqE) and petroleum ether extract (PEE) of *S. marianum* seeds were administered @ 1000 mg/kg body weight orally daily from 3rd to 7th day of experiment to different groups of albino rabbits paracetamol (500 mg/kg, once orally on 1st day) was administered. The PEE of *S. marianum* seeds significantly ($P<0.05$) decreased the enhanced levels of serum alkaline phosphatase (SAP), serum arginase (SARG), glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) due to paracetamol administration. However, the AqE of *S. marianum* seeds significantly counteracted the paracetamol induced elevation of SARG and SGPT. The paracetamol mediated reduction in the levels of serum total protein (STP), and its fractions (viz., albumin, globulin and albumin/globulin ratio) was elevated by *S. marianum* seed extracts. The results suggest that the extracts of *S. marianum* seeds may protect the liver from damaged by various hepatotoxicants.

KEY WORDS: Paracetamol, hepatotoxicity, *Silybum marianum* seed, silymarin, hepatogenic effect, rabbit.

INTRODUCTION

Many infections, contaminants, chemicals and drugs (including paracetamol) have been reported to cause hepatotoxicity, resulting into alterations in liver function tests (LFTs) including enzymes and proteins (Pandey 1980; Pandey et al., 2008). *S. marianum* (milk thistle) belonging to Compositae family, is an edible plant, native to the Mediterranean and grows throughout Europe, North America, India, China, South America, Africa and Australia. *S. marianum* seed contain silymarin, a flavonolignan (polyphenolic fraction). Silymarin flavonoid has been reported to be antihepatotoxic agent against toxicity caused by various agents. *S. marianum* seeds and silymarin may restore the LFTs and regenerate hepatic tissues (Kshirsagar et al., 2009; Pandey 1980; Pandey and Sahni, 2011; Vogel, 1977). The *S. marianum* seeds and silymarin possess antioxidant, immunomodulatory, anticancer, antiinflammatory, antihepatotoxic (or liver regenerating), antifibrotic, antilipid peroxidative, membrane stabilizing activities/mechanisms, etc. Its effectiveness against multiple disorders makes it a very promising drug of natural origin (Pandey and Sahni, 2011). In the present study, paracetamol was taken as a standard hepatotoxic drug to induce the hepatotoxicity in rabbits. In the present study the hepatogenic effect of *Silybum marianum* seed was evaluated on the basis of alterations in some biochemical parameters viz., SAP, SARG, SGOT, SGPT, and STP and its fractions.

MATERIALS AND METHODS

Sixteen healthy inbred albino rabbits of either sex (1.0-1.5 kg) equally divided into 4 groups were kept in colony cages under identical managemental conditions. They were fed on uniform balanced diet and drinking water *ad libitum*. Powdered paracetamol (acetaminophen) was obtained from Duphar-Interfran Ltd., Bombay, India. The *S. marianum* seeds powder was procured from Regional Research Laboratory (Govt. of India), Jammu-Tawi, J & K. The extraction of powdered *S. marianum*

seeds was done as per the methods used by Pandey (1980). *S. marianum* seeds PEE was prepared with petroleum ether (60-80°C), and the AqE was prepared with distilled water before use. During experiment, the required quantity of paracetamol solution was prepared in normal saline containing 20% propylene glycol. To make complete suspension of the PEE of *S. marianum* seeds, it was dissolved in distilled water containing 2-3 drops of polysorbate-80.

The rabbits of group 1 (normal) were given saline alone, whereas the rabbits of groups 2 to 4 were administered paracetamol @ 500 mg/kg, once orally on the 1st day of experiment (1st day of paracetamol administration). After 48 hr of paracetamol administration, the AqE and PEE of *S. marianum* seeds were administered @ 1000 mg/kg, orally, daily for 5 days from 3rd to 7th day to groups 3 and 4, respectively. The rabbits of group 2 which did not receive *S. marianum* extracts, served as experimental control and they were kept under study for 7 days after paracetamol administration. After the experiment, the biochemical study was conducted.

Blood was collected from ear veins of the rabbits of all the groups on the 1st day and 8th day, respectively. The blood was centrifuged for 10 minutes at 2500 rpm and serum was separated. The activity of SAP was measured in terms of KAU (mg/phenylphosphate/30 min) as per the method described by King and King (1954). The activity of SARG expressed in terms of IU/L of serum was assayed as per the method of Mia and Koger (1978). The activities (U/ml of serum/minute at 37^0C) of SGOT (aspartate aminotransferase, AST) and SGPT (alanine aminotransferase, ALT) were estimated according to the method of Reitman and Frankel (1957). However, the concentrations (g/dl) of STP and its fractions, viz., albumin, globulin and albumin/globulin (A/G) ratio were determined as per the method of Doumas et al. (1971). The kits of Span Diagnostics Pvt. Ltd., Udhna, Gujrat were used for estimation of SAP, SGOT, SGPT, SAP, and serum total proein (STP) and its fractions. For SARG test, the reagents were prepared in the lab. The biochemical data were analyzed statistically to calculate the mean and standard error (SE). To find out the significance of difference among different groups, Duncan's new multiple range test (ANOVA) at $P<0.05$ (5% level of significance) in completely random design was employed.

RESULTS AND DISCUSSION

The average values (Mean±SE) of SAP, SARG, SGOT and SGPT estimated in groups 1 to 4 of rabbits are shown in Table 1. The activities of these enzymes recorded in group 1 (normal) were increased significantly ($P<0.05$) after treatment with paracetamol in group 2. Further, the SAP and SGOT activities of groups 2 to 4 also differed significantly with each other. However, the SARG and SGPT activities of groups 2 and 3 both differed significantly from group 4, but the activities of groups 2 and 3 did not differ significantly with each other. The non-significant decrease in the activities of all these enzymes appeared to decline towards normal after treatment with *S. marianum* seeds PEE in group 4. The average values (Mean±SE) of STP and its fractions (viz., albumin, globulin and A/G ratio) recorded in groups 1 to 4 of rabbits are presented in Table 2. As compared to group 1, there was a significant ($P<0.05$) decrease in the values of STP and its fractions except A/G ratio in group 2 treated with paracetamol alone. However, the decreased values of STP, albumin and globulin recorded in group 2 were significantly increased by administration of PEE of *S. marianum* seeds in group 4, and these values returned to normal.

The values of biochemical profile of group 1 viz. SAP, SARG, SGOT, SGPT, STP were corresponds with previous workers. Jain et al. (2007); King and King (1954); Mia and Koger (1978) Reitman and Frankle (1957) and Doumas et al. (1971).

In the present study, the alteration in serum enzymes and proteins after paracetamol administration is indicative of hepatotoxicity (liver injury). The alterations in different LFTs during paracetamol induced hepatotoxicity have also been reported earlier by Pandey (1980). Paracetamol injures the hepatic cells and alters the normal levels of serum enzymes and proteins by depleting protein

Table 1: Effect of *Silybum marianum* seeds on SAP, SARG, SGOT and SGPT enzymes on paracetamol induced hepatotoxicity in rabbit

Group#	Treatment*	Dose (mg/ kg, oral)	Day of experi-ment	SAP activity Mean±SE** (KAU)	SARG activity Mean±SE** (IU/L)	SGOT activity Mean±SE** (U/ml)	SGPT activity Mean±SE** (U/ml)
1	Saline (Normal group)	-	1st	7.2±0.12^{c}	3±1.22^{d}	29±1.08^{c}	27±0.41^{d}
2	Paracetamol	500	8th	11.6±0.51^{a}	36±1.41^{a}	64±1.83^{a}	53±1.29^{a}
3	Paracetamol	500	8th	11.0±0.13^{a}	24±1.63^{b}	60^{b}±0.82^{a}	40±0.71^{b}
	S. marianum (AqE)	1000					
4	Paracetamol	500	8th	8.9±0.20^{b}	9±1.29^{c}	37±0.41^{b}	35±0.41^{c}
	S. marianum (PEE)	1000					

\# Number of rabbits in each group = 4.

* Paracetamol administered only once on the 1st day, followed by S. marianum extract daily from 3rd to 7th day of experiment.

** Means with same superscript do not differ significantly (Duncan's new multiple range test at P=0.05).

Table 2: Effect of *Silybum marianum* seeds on serum total protein (STP) and its fractions on paracetamol induced hepatotoxicity in rabbit

Group#	Treatment*	Dose (mg/ kg, oral)	Day of experi-ment	STP concentration Mean±SE** (g/dl)	Albumin concentration Mean±SE** (g/dl)	Globulin concentration Mean±SE** (g/dl)	A/G ratio Mean±SE** (g/dl)
1	Saline (Normal group)	-	1st	5.92±0.08^{a}	4.30±0.03^{a}	1.62±0.04^{a}	2.65±0.04^{a}
2	Paracetamol	500	8th	5.03±0.07^{c}	3.60±0.04^{c}	1.43±0.03^{c}	2.52±0.03^{ab}
3	Paracetamol	500	8th	5.16±0.03^{bc}	3.72±0.08^{c}	1.44±0.06^{bc}	2.58±0.17^{a}
	S. marianum (AqE)	1000					
4	Paracetamol	500	8th	5.59±0.03^{ab}	3.99±0.09^{b}	1.60±0.09^{ab}	2.49±0.23^{ab}
	S. marianum (PEE)	1000					

\# Number of rabbits in each group = 4.

* Paracetamol administered only once on the 1st day, followed by S. marianum extract daily from 3rd to 7th day of experiment.

** Means with same superscript do not differ significantly (Duncan's new multiple range test at P=0.05).

synthesis (Pandey 1980; Pandey et al., 2008). Aqueous extract of *S. Marianum* did not alter the toxic effect of paracetamol.

The results indicated that most of the LFTs improved and returned towards normal level after treatment with extracts of *S. marianum* seeds.

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