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STUDY ON THE HEPATOPROTECTIVE EFFECT OF *SILYBUM MARIANUM* EXTRACT ON EXPERIMENTAL POISONING BY PARACETAMOL IN NMRI ALBINO MICE

Mădălina Elena Scarlat [1,*], Monica Bucă [1], Magdalena Cristina Zagardan [1], Alina Păunescu [1], Cristina Maria Ponepal [1], Monica Popescu [1], Alexandru Gabriel Marinescu [1]

[1] University of Piteşti, Faculty of Sciences, Physical Education and Informatics, Târgu din Vale St. 1, 110040, Piteşti, Romania

Abstract

Hepatoprotection is a matter of worldwide interest, since liver diseases are common, and liver transplant has increasedover the past years, drastically reducing the number of people able to meet the criteria for such a transplant. The experimental research in this paper aimed at evaluating the hepatoprotective capacity of Silybum marianum species (thistle). In order to fulfil the purpose of the paper, thistle was administrated as hydr alcoholic extracts to animals, i.e. albino mice-NMRI strain. We used paracetamol solution for infusion known as Perfalgan (Bristol-Myers Squibb). The dose used was 400mg/kg/bod y substance, and we administrated ethanolic extracts of Sylibum marianum after the paracetamol poisoning. We conducted the research using biochemical methods and techniques, potential structural and functional changes occurred in the experimental animals' internal organs poisoned with perfalgan then treated with Silybum marianum plant extract.

Keywords: Hepatoprotection, Paracetamol, Silybum marianum.

1. INTRODUCTION

The goal of the experimental research described in this paper was to evaluate the hepatoprotective capacity of Silybum marianum (thistle) species when there is experimental poisoning by Paracetamol, a non-steroidal anti-inflammatory medicine often used for its analgesic effect.

Paracetamol is a widely used analgesic and antifever medicine. It is used and can be purchased with or without prescription in many countries (Kittisupamangkol, 2009).

There have been various studies and experiments on ethanolic extracts from different plants to demonstrate their beneficial effects.

The study called "Extract of okra lowers blood glucose and serum lipids in high-fat diet-induced obese C57BL/6 mice" (Fan et al., 2014) showed that okra (also known as gambo) considerably lowered blood glucose level, as well as serum insulin; also, an improvement as regards glucose tolerance was found out in the obese mice. Serum triglycerides and cholesterol values also reported significant decrease, while hepatic morphology was improved significantly in the okra- treated mice.

The pomegranate peel's hepatoprotective effect was tested by Ashoush et al. (2013) in the paper titled "Antioxidant activity and hepatoprotective effect of pomegranate peel and whey powders in rats". The study highlighted the hepatoprotective effect in Wistar rats fed for 28 days with a mixture

*Corresponding author, E-mail address: gabriel_mda8487@yahoo.com

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of pomegranate peel and whey powder as compared to the control group, which consisted of rats experimentally poisoned by carbon tetrachloride. They managed to restore the biochemical parameters and improved liver histological change. Thus, it could be concluded that whey powder could be incorporated with the pomegranate peel for consumption by people suffering from liver diseases.

Nithianantham et al. (2011) showed, in the work called "Hepatoprotective effect of Clitoria ternatea leaf extract against paracetamol induced damage in mice" by the experimental results of induced hepatic toxicity that the mice which had been treated with Clitoria ternatea leaf extract (200 mg/kg) registered significant decrease in SLT, ASTL and bilirubin levels. They also found out that therapy using Clitoria ternatea leaf extract is protective against histopathological damage. Histological studies supported the biochemical results, thus maximum improvement of the histo-architecture was found out. This paper deals with the influence of Silybum marianum species while under paracetamol toxicity in experimental conditions in NMRI mice.

The thistle is part of the Asteraceae family, and it is a plant used as remedy to treat liver and gall bladder diseases. The fruits picked in August are used in phytotherapy, since they are rich in saponoside, silymarin, fitomeli, fumaric acid, all these favouring liver cells regeneration and heightening liver's property of fighting –off infections.

Silymarin has been and is, thanks to its unique complex of substances – containing silibinin, silidianin and sillicristin, the subject of decades of research on its beneficial properties.

Silymarin has hepatoprotective properties and is used to treat various liver diseases (Elmowafy et al., 2013).

There are various studies showing that silymarin comprises a strong antioxidant activity (Simeonova et al., 2013) and protective effects against liver toxicity induced by a great variety of agents by inhibiting lipids peroxidation (Bosisio et al., 1992; Binda et al., 2001). The fact that a higher total phenol content contributes to the extracts' antioxidant activity is well known (Yuan et al., 2014), while antioxidant activity has also been tied to the hepatoprotective effect of certain plant extracts (Gu et al., 2014; Yuan et al., 2014).

In the work titled "Hepatoprotective effect of Silymarin (Silybum marianum) on Hepatotoxicity induced by Acetaminophen in Spontaneously Hypertensive Rats", Freitag A.F. et al. monitored how silymarin impacts hypertension and the hepatic state induced by Acetaminophen (paracetamol) in spontaneously hypertensive rats. Normotensive or hypertensive animals were treated by paracetamol (3 g/kg body orally), some of them having been previously treated with Silybum marianum extract. Plasmatic levels of the liver function's markers were determined 12 hours after the paracetamol had been administered: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), glucose (GLU), Gamma Glutamyl - Transferase (γ-GT) and alkaline phosphatase (ALP).

Liver disorders were assessed using histological studies.

The liver samples were used to determine myeloperoxidase activity (MPO) and nitric oxide (NO) and they were also sliced for histological tests.

Further to *Silybum marianum* treatment, the AST, ALT levels were restored to normal levels in all mice batches to have been administered Acetaminophen.

It is common knowledge that hepatotoxic medicines, such as paracetamol, cause significant increase in the serum level of enzymes, such as ALT (Alanine Aminotransferase), AST (aspartate Aminotransferase), ALP (alkaline phosphatase) and bilirubin, which is indicative of a significant hepatocellular lesion (Green, 2010). When there is hepatopathy, these enzymes pass to the blood flow, according to the liver injury degree.

*Corresponding author, E-mail address: gabriel_mda8487@yahoo.com

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Low concentrations of these enzymes following administration of *Silybum marianum* plant extracts could be partially due to the presence of chemical constituents in the extract. Plasmatic decrease in such marker enzymes could also be due to the anti-hepatotoxic effect of the *Silybum marianum* extract.

2. MATERIALS AND METHODS

Swiss- albino mice- NMRI strain were used for this research, weighing over 30g; they were housed under standard lab conditions, into metal cages, receiving special ration and water ad libitum, in a controlled, 12 hours light/12 hours dark Circadian rhythm, and an ambient temperature of 22±2°C. The animals were provided by SPF Animal House, Băneasa Resort, Cantacuzino Institute of Bucharest.

The experiment consisted of four experimental batches, each batch being made up of 6 mice, as follows: a control group, a batch of mice intraperitoneally injected by 0.4mg of Perfalgan, a batch of mice injected by 0.4 mg Perfalgan and treated by gavage using 0.2 ml thistle extract for 14 days and a batch treated only with thistle plant extract in a concentration of 0.25 ml.

Table 1. Experimental batches

Batch		Intraperitoneal injection	Gavage
Control- group (6 mice)			
Paracetamol- one batch		x	
Thistle	Thistle extract+ Paracetamol- one batch	x	x
	Thistle batch- one batch		x

The thistle plant extracts were obtained using two processing cycles: primary and advanced; ethyl alcohol was used as solvent in various concentrations (Lupuleasa et al., 2005).

To complete the symptomatic picture of the toxic effect of Paracetamol, studied and demonstrated by various experimental works, cholesterol and triglycerides values were determined in this research. Thus, blood samples were collected by venipuncture at the basis of mice's tail, and the above-mentioned indices were determined.

3. RESULTS AND DISCUSSIONS

Figure 1 shows how cholesterol can vary while under the influence of Paracetamol administered in 400 mg/kg/body concentration, but also under the influence of *Silybum marianum* extract, administered into two doses of 0.25 mg for seven days.

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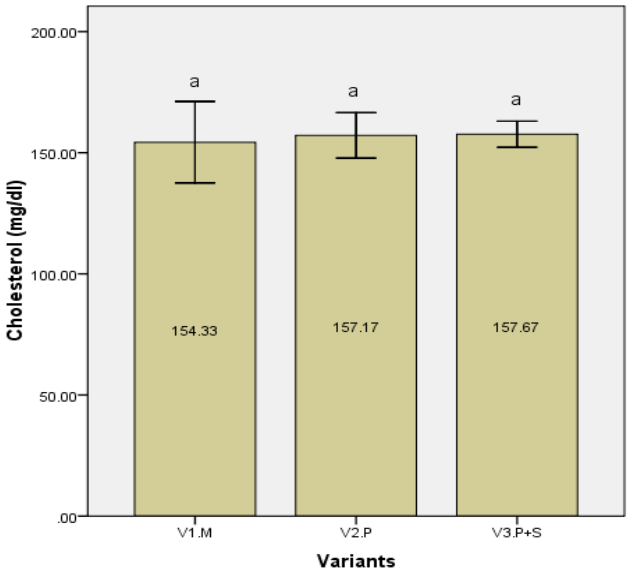

Figure 1. Silybum marianum extract's influence over cholesterol for Paracetamol-induced toxicity (V1.M = control variant; V2.P =Paracetamol batch 400mg/kg body; V3.P+S = Paracetamol batch 400 mg/kg body treated by Silybum marianum extract) (letters represent Duncan's range test interpretation: different letters show significant differences, p<0.05)

No significant changes have been found as regards this physiological index further to the administration of Paracetamol, and *Silybum marianum* extract reported no significant effects of cholesterol values after the two doses of 0.25 mg were administered.

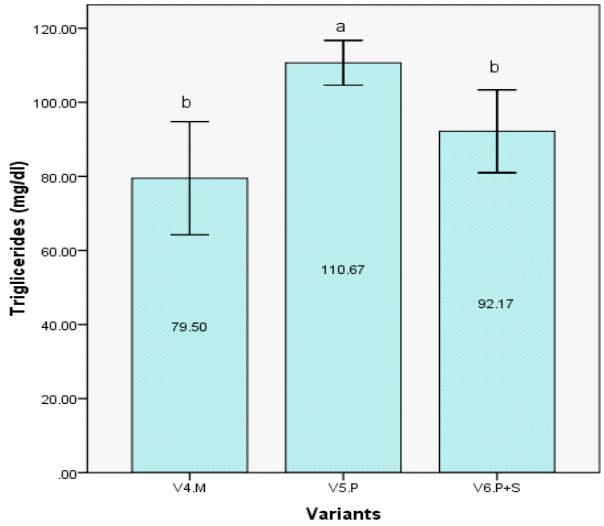

Figure 2. Silybum marianum extract's influence over triglycerides for Paracetamol-induced toxicity (V1.M = control variant; V2.P =Paracetamol batch 400mg/kg body; V3.P+S = Paracetamol batch 400 mg/kg body treated by Silybum marianum extract) (letters represent Duncan's range test interpretation: different letters show significant differences, p<0.05)

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Further to Paracetamol's administration in 400 mg/kg/body concentration, significant increase was noticed in triglycerides values after 168 h from administration and gradual return of this physiological index to values close to normal limits after two doses of *Silybum marianum* extract have been administered in a concentration of 0.25mg.
Most specialist works consider Paracetamol's toxic effect on liver level. Thus, Erfidan et al. (2016) highlighted in the work titled "*Pomegranate effect over Paracetamol-induced liver damage in mice"* significant increase in serum levels of AST, ALT, TP and TG. Also, when administered in high doses, Paracetamol causes centrilobular hepatic necrosis (Mitchelli et al., 1973; Alfino et al., 2006), and another study shows that in doses of 300mg/kg/body or higher, it causes severe acute hepatic necrosis (Douidar et al., 1985).
Metabolic and biochemical events leading to paracetamol toxicity have been described by various studies over the years; however, the exact mechanism of cell damage is still unknown (Larson, 2007).

4. CONCLUSIONS

Paracetamol administration in a concentration of 400 mg/kg/body determines significant increase in triglycerides value seven days after administration.
Silybum marianum extract, administered into two doses at a concentration of 0.25 mg for 7 days has no significant effect on cholesterol; instead, it leads to significant decrease in triglycerides, close to normal limits at the Paracetamol-treated batch.

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