Evaluation of Protective and Antioxidant Activity of MilkThistle on Paracetamol-Induced Toxicity in Rats

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Abstract: Paracetamol is a common analgesic and antipyretic drug which is safe in therapeutic doses but can produce life-threatening hepatic and renal damages with toxic doses. The current study was designed to investigate the protective effects of aqueous extract of milk thistle against paracetamol-induced toxicity in female rats. A total of 24 rats were used for the study. The rats were grouped into four with sex rats in each group. Group I was the control, group II received milk thistle at a dose of 500 mg / kg body weight for 60 days. Group III received paracetamol at a dose of 200 mg / kg body weight / ml for 60 days, and group IV received paracetamol plus milk thistle for 60 days. The results revealed that administration of paracetamol to rats induced marked disturbance of lipid profile, hepatic and renal functions, characterized by increasing in the levels of cholesterol, triglyceride, LDLc, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin , uric acid, blood urea, serum creatinineand decreasing super oxide dismutase (SOD), catalase (CAT), activities and reduced glutathione (GSH) levels. Histopathological changes showed that paracetamol caused damages to liver. Oral co-administration of milk thistle with paracetamol significantly decreased the level of liver enzymes (ALT, AST), total bilirubin,uric acid, blood urea and creatinine. Milk thistle treatment also resulted in a significant increase in CAT, SOD and GSH. These results clearly show the antioxidant and protective property of milk thistle.
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Key words: Paracetamol, milk thistle, antioxidants, hepatotoxicity, rats.

1.Introduction

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury (Friedman *et al.,* 2003) and it is the most common reason for a drug to be withdrawn from the market. Drugs are also, a common source of acute renal injury and cause approximately 20 percent of community- and hospital- acquired episodes of acute renal failure (Bellomo, 2006). Renal toxicity should not be confused with the fact that some medications have a predominantly renal excretion and need their dose adjusted for the decreased renal function. Paracetamol is a common analgesic and antipyretic drug which is safe in therapeutic doses but can produce life-threatening hepatic and renal damages in man, rats and mice with toxic doses (Abraham, 2005). Protection against paracetamol – induced hepatoreno-toxicity has been used as a test for potential protective activity by several investigators (Visen *et al.,*1993). A number of drugs or chemicals such as melatonin, vitamin E and N-acetyl-cysteine have been used to prevent paracetamol -induced hepatic and renal injury (Sener*et al.,*2003). Increased use of synthetic drug therapy leads to many side effects and undesirable hazards. Therefore there is a worldwide trends to return to natural resources, which are culturally acceptable and economically viable. It was shown that milk thistle (*Silybummarianum*) has beneficial effects on hepatotoxicity (Pepping, 1999). Reports showed that milk thistle promote DNA polymerase, stabilize all membranes, inhibits free radicals and increases glutathione concentration, so it could protect liver from hepatotoxic agents. Silibinin is able to stimulate the activity of the DNA-dependent RNA polymerase I and causes an increase in rRNA synthesis. It accelerates formation of intact rRNA polymerase with resultant formation of new hepatocytes (Valenzuela *et al.,*1998). Treatment with milk thistle has been usual since 2000 years ago and it is mentioned as a hepatoprotective agent (Ross, 2008). Milk thistle is found in many areas all around the world and is cultured in North and South parts of Iran. This drug is absorbed via the gastrointestinal tract; the maximum blood level is reached after 2-4 hours. The half-life of the drug is six hours. About 80% of milk thistle is secreted into the bile and its bioavailability depends on its formulation (Weyhenmeyer *et al.,* 1992). Sylibin is the most effective agents in milk thistle and is known as an

antioxidant and hepatoprotective agent. Its concentration in bile is 60 times greater than the blood.

The present study was designed to assess whether the toxic effects caused by administration of paracetamol could be prevented or ameliorated by treatment with milk thistle.

2.Materials and Methods

Chemicals:

Paracetamol

Plant Material:

Milk thistle was purchased from Cairo local market. It was grinded in a blender. The powder of sample was kept in a polyethylene bags until use.

Animals:

Adult female white rats weighing (125-140g) were used in this study. All animals were housed in plastic cages and kept under the same laboratory conditions of temperature (25±2°C) and lighting (12:12hr light: dark cycle), for one week prior to starting the experiments. The rats were provided *ad libitum* with tap water and fed with standard diet (Table1).

Table (1): Composition of the standard diet.

Ingredients	g/kg diet
Casein	200
Corn starch	497
Sucrose	100
Vitamin mixture	020
Mineral mixture	100
Corn oil	050
Cellulose	030
Methionine	003

Experimental Design:

The animals were divided randomly into four groups of sex rats each. Paracetamol was prepared by dissolving in distilled water and administered orally at the dose of 200 mg/kg body wt. (Vidhya and Mary, 2009), while the milk thistle was administered by oral gavage at a dose of 500 mg/kg body wt. (Shati and Elsaid, 2009). The four groups were as follows:

Group I: Vehicle (distilled water) treated rats were received daily dose of distilled water for 60 days and served as control.

Group II: Rats received milk thistle (500 mg/kg b. wt /day, orally) for 60 days.

Group III: Rats received paracetamol (200 mg/kg b. wt /day, orally) for 60days.

Group IV: Rats received paracetamol as in group III and after 30 min milk thistle was administered as in group II for 60 days.

Daily food intake and weekly body weight were recorded. The percentage of the daily changes in body weights were calculated according to the following formula:

Change in body weights (%) = 100 X (Final weight – Initial weight) / Initial weight

Food efficiency ratio (FER) was calculated at the end of experiment as following: FER= Body weight gain (gm) / Food intake (gm)

Biochemical analysis:

At the end of the experimental period, the rats were anaesthetized by diethyl ether and sacrificed. Blood samples were collected in clean test tubes and left for coagulation then centrifuged at 3000 rpm for 15 minutes to obtain serum. Total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDLc) in serum were determined according to the method of **Richmond (1973), Fassati and Principe (1982) and Gordon (1977),** respectively. Low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLC) was calculated using the method of **Hatch and Lees (1968)** and**Friedewald (1972),** respectively. Atherogenic indices were obtained by dividing TC / HDLc or dividing LDLc / HDLc according to **Castelli and levitar (1977)**. Bilirubin, Serum alanine aminotransferase (ALT) and aspartate aminotransferase enzymes (AST) were determined according to the method described by**Jendrassik (1938)**, **Reitman and Frankel (1957)**. Serum urea and creatinine were determined according to the method described by **Fawcett and Scott (1960) and Bartles** *et al.* **(1972),** respectively. GSH (**Beutler*et al.*, 1963**), SOD**(Nishikimi*et al.*, 1972)**, and CAT **(Aebi, 1984)** analysis**.**

Statistical analysis:

All obtained data were statistically analyzed by SPSS computer software. The calculated data accorded by analysis of variance ANOVA and follow up LSD (SPSS) Computer programvariation**.**

3.Resultsand Discussion

Effect of administration of paracetamol alone or with milk thistle on body weight, weight gain, food intake and food efficiency ratio.

Data in Table (2) shows that the body weight of all the rats groups increased at the end the experiment. The highest weight gain was noticed in the groups received milk thistle (42.8g and 48.0g), which revealed higher values than paracetamol control group (30.8g).On the other hand, the results

show that food efficiency ratio of paracetamol group rats was 2.2 and 2.5 in control group whereasthe groups received milk thistleonly or with paracetamolwere 3.1 and 3.5, respectively.

Effect of administration of paracetamol alone or with milk thistle on lipidsprofile of experimental rats.

The results recorded in Table (3) showed significant decreases in TC,TG, LDLc and VLDLc and significant increase in HDLc in groups received milk thistle groups only and with paracetamol compared with paracetamol group. These results agreed with (**Zima *et al.*,1998**) who showed that milk thistle prevents atherosclerotic plaque formation in aorta. It has been shown that the cisplatin and cyclosporine side effects reduced when milk thistle was administered in mice. Administration of silibinin does not reduce plasma levels of cholesterol in normal rats; however, it reduces total phospholipid levels. Biliary cholesterol and phospholipid concentrations in rats are also slightly reduced. Silymarin-induced reduction of biliary cholesterol and phospholipids in both rat and human may be in part due to decreased liver cholesterol synthesis. Silymarin could represent a novel agent in the prevention and therapy of hypercholesterolemia and atherosclerosis. **(Das *et al.*, 2008)** The antihypercholesterolemic effect of silymarin was associated with liver cholesterol reduction (**Krecman *et al.*, 1998**), which improves cholesterol uptake from blood.

Table (2): Effect of administration of paracetamol alone or with milk thistle on body weight, weight gain, food intake and food efficiency ratio.

Groups	Initial weight(g)	Final weight(g)	weight gain(g)	weight gain %	Daily food intake	Food fficiency ratio
Control -	130.80±4.49	165.80±12.69	35.0±9.27	26.7±0.07	13.66±0.63	2.50±0.61
Milk thistle	131.0±3.93	173.8±5.71	42.8±4.32*	32.6±0.03**	13.28±0.47	3.18±0.37**
Paracetamol	131.80±5.80	162.6±14.44	30.8±9.03	23.3±0.05	13.5±0.50	2.24±0.57
Paracetamol+Milkthistle	132.4±5.59	180.4±12.05	48.0±9.43**	36.2±0.06**	13.52±0.47	3.51±0.64**

Each value is the mean± SD of 5 rats. The values in the column with the same superscript are not significant different at P<0.05.

Table (3): Effect of administration of paracetamol alone or with milk thistle on lipidsprofile.

Groups	T.C.(mg/dl)	TG(mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control -	68.0±9.08	74.6±9.8	32.0±1.5	15.6±5.7	14.9±1.9
Milk thistle	76.80±5.63***	76.0±11.78***	40.20±4.96***	18.60±2.96***	15.20±2.35***
Paracetamol	126.4±14.75	175.6±34.76	28.0±2.34	58.8±17.97	35.12±6.95
Paracetamol+Milkthistle	80.0±9.82***	79.20±11.98***	38.0±4.69**	20.20±4.81**	15.84±2.39***

TC:Total cholesterol TG:TriglycerideHDL:High density lipoprotein cholesterol
LDL:Low density lipoprotein cholesterol VLDLc:Very low density lipoprotein cholesterol
Each value is the mean± SD of 5 rats.
The values in the column with the same superscript are not significant different at *P*<0.05.

Atherogenic indices of experimental rat groups at the end of the study

Belonging to the ratio of cholesterol/HDL-cholesterol and LDL/HDLc, it was found that all treated groups with milk thistle revealed significant decreases in Cholesterol/HDL-c and LDL/HDLc ratio as compared to paracetamol treated group .Silibinin has been found to have beneficial effects oncholesterol levels. It is thought that its antioxidantactivity helps to protect cholesterol-transportinglipoproteins. It has also been found to influencecholesterol metabolism, and can block a key enzymeinvolved in the synthesis of cholesterol. In rats, milkthistle seed oil was found to reduce serum totalcholesterol and triglyceride levels by 84 and 60%. (**Greenlee*et al.*, 2007**).Free radicals are recognized to have an importance in mechanisms of many pathological processes including atherosclerosis lipid peroxidation of LDL (**Mennen *et al.*, 2004**). It has been shown that flavonoids such as silymarin are large group naturally occurring antioxidants that could inhibit lipid peroxidation of LDL by scavenging free radicals, after the administration of silymarin, the antioxidant enzyme activities reversed to near normal (**Kiruthiga *et al.*, 2007**).

Table (4): Atherogenic indices of experimental rat groups at the end of the study

Groups	Cholesterol/HDLc	LDL/HDLc
Control -	2.1±0.28	0.47±0.17
Milk thistle	1.88±0.28***	0.46±0.08***
Paracetamol	4.50±0.96	2.06±0.59
Paracetamol+ Milkthistle	2.11±0.41***	0.54±0.18***

Each value is the mean± SD of 5 rats.

The values in the column with the same superscript are not significant different at *P*<0.05.

Effect of administration of paracetamol alone or with milk thistle on serum ALT, AST, Urea, Creatinine, Uric Acid and Bilirubin of different experimental rat groups at the end of the study

The extent of paracetamol induced hepatotoxic effect was assessed by the level of released cytoplasmic enzymes such asALT,AST.The activities of tranaminases,Urea,Creatinine,Uric Acid and the level of total Bilirubin are shown in Table5. The level of ALT, AST, Urea,Creatinine, Uric Acid and Bilirubin increased in paracetamol –treated rats. Co-treatment with milk thistle only or withparacetamol reduced the concentration of ALT, AST, Urea,Creatinine, Uric Acid and total Bilirubin significantly in comparison with paracetamol –treated group. Acute large doses or chronic use of paracetamol associated with hepatotoxicity and nephrotoxicity in humans as well as animals (**Schnellman, 2001**). These results agreed with**Ghaffari *et al.***(2011) who reported thatmilk thistle extract protects liver against methotrexate hepatotoxic effects. Other studies also showed thatmilk thistle extract prevents kidney injury due to methotrexate in rat.Milk Thistle extract significantly prevents liver damage due tomethotrexate in rat, Silymarin, an extract from seeds and fruits of milk thistle (*Silybummarianum*), is a mixture of flavonoid isomers such as silibinin, isosilibinin, silidianin, and silichristin. The seeds of this plant have been used in Europe for many centuries for the treatment of liver and gall bladder dysfunctions (**Schulz *et al*., 2004**). In the treated group (milk thistle group) silymarin dramatically reduced the elevation of ALT activity and increased the albumin level. As it is well known, Silymarin is frequently used in the treatment of liver diseases where it is capable of protecting liver cells directly. It does this through stabilizing the cell membrane by preventing liver glutathione depletionand inhibiting lipid peroxidation (**Mira *et al*., 1994**).The stimulatory effect ofsilymarin on liver regeneration was observed only in damaged livers indicating that silymarin increasesregeneration potency of damaged liver tissues. The pharmacological propertiesof silymarin involve regulating cell membrane permeability and integrity, inhibiting leukotriene, scavenging reactive oxygen species,

Table (5): Serum ALT, AST, urea and creatinine of different experimental rat groups at the end of the study

Groups	ALT	AST	Urea	Creatinine	Uric Acid	Bilirubin
Control -	32.6 ±5.85	18.40±4.8	26.2±3.03	0.47±0.10	2.8±0.38	0.45±0.03
Milk thistle	32±8.39**	17.4±3.50***	26.80±3.2*	0.64±0.15***	2.94±0.35**	0.53±0.08**
Paracetamol	56.80±8.2	28.4±2.96	34.0±6.32	1.86±0.43	4.04±0.58	1.0±0.32
Paracetamol+Milkthistle	31.4±7.4***	16.0±3.93***	22.4±3.6**	0.48±0.11***	2.54±0.6**	0.55±0.09**

ALT:alanine aminotransferase enzymes AST:aspartate aminotransferase enzymes

Each value is the mean± SD of 5 rats.The values in the column with the same superscript are not significant different at P<0.05.

Effect of administration of paracetamol alone or with milk thistle on serum CAT, SOD and GSH of different experimental rat groups at the end of the study

Table (6) showed thatparacetamol with milk thistle groups showed (p<0.01) a significant increase in CAT and a significant increase in SOD at (p<0.001). Milk thistle groups also showed a significant increase in GSH compared with paracetamol treated group.This observation is in agreement with that found by (**Kshirsagar *et al*., 2009**) who found that Silymarin, an extract from seedsand fruits of milk thistle (*Silybummarianum*), is a mixture of flavonoidisomers such as silibinin, isosilibinin,silidianin, and silichristin. Silymarin has liver regenerativeeffects by stimulating the enzyme knownasRNA polymerase in the nucleus ofliver cells. This results in increase ofribosomal protein synthesis which helps toregenerate hepatocytes (**Gruenwald, 2004**). The ability of silymarin to protectagainst oxidative stress-inducedhepatocellular damage (such as lipidperoxidation of membranes andsubsequent membrane degradation) isassociated with its free radicalscavenging properties and its ability toenhance endogenous antioxidantdefences, such as those mediated by Super oxide dismutaseor the glutathione system (**Schuppan*etal*., 1999)**Silymarin has been recognized as an excellent antioxidant,scavenging free radicals (reactive oxygen species) and inhibiting lipid peroxidationthereby protecting cells against oxidative stress. It augments the non-enzymatic and enzymatic antioxidant defense systems of cells involving reduced glutathione,superoxide dismutase and catalase. It can protect the liver, brain, heart and other vitalorgans from oxidative damage for its ability to prevent lipid peroxidation andreplenishing the reduced glutathione levels. Silibinin exhibits membrane protectiveproperties and it may protect blood constituents from oxidative damage. (**Kshirsagar*et al.*, 2009**)

Table (6): Effect of administration of paracetamol alone or with milk thistle on serum CAT, SOD and GSH of different experimental rat groups at the end of the study

Groups	CAT	SOD	GSH
Control -	590.0±118.32*	0.54±0.12	6.42±1.04
Milk thistle	694.0±104.78**	0.66±0.12***	6.66±0.69**
Paracetamol	344.0±137.2	0.28±0.05	4.22±1.54
Paracetamol+Milkthistle	652.0±186.86**	0.63±0.08***	6.28±0.84*

Histopathological Results of Liver:

Microscopically, Liver of rat from control group (-ve) showing no histopathological changes (Fig.1). Moreover, Liver of rat from control group(-ve) consumed milk thistle showing normal hepatocytes (Fig.2). Meanwhile, liver of rats from group treated byparacetamol showing cytoplasmic vacuolization of hepatocytes, hepatoportal blood vessel and collagen fibers deposition in portal triad and cytoplasmic vacuolization of hepatocytes and focal hepatic haemorrhage (Fig.3,4,5) respectively, Apparent slight vacuolation of hepatocytes and slight cytoplasmic vacuolations of hepatocytes was noticed in liver of rats from paracetamol and milk thistle group(Fig .6,7) respectively. **El-Shafeey *et al.* (2012)** found that the rats fed on silymarin after Fumonisin B1 administration showed less signs of amelioration. They also stated thatalthough mild cloudy cells, pyknotic nuclei, infiltration of lymphocytes, ruptured boundaries of vascular wall and appearance of larger sinusoids are still found; the regeneration of hepatocytes, activation of Kupffer cells, and rearrangement of hepatocytes displaying architecture with less appearance of necrotic areas.

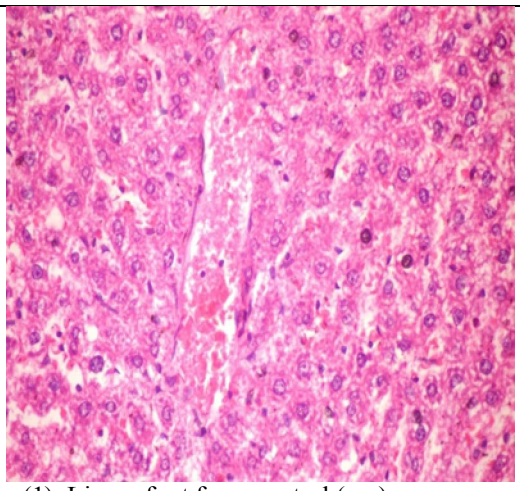

Fig. (1): Liver of rat from control (-ve) group

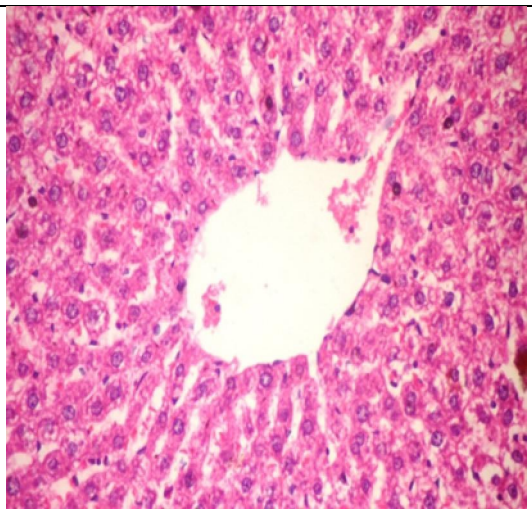

Fig (2): Liver of rat from control (-ve) and milk thistle group

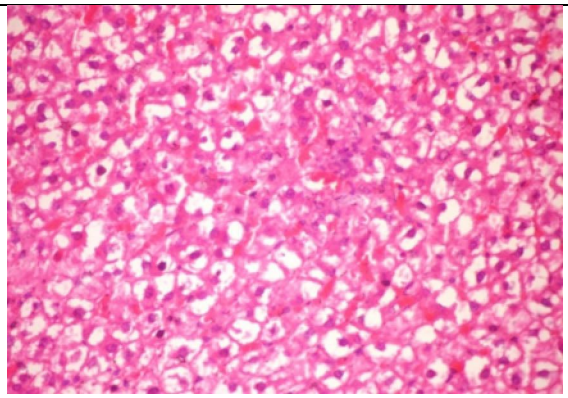

Fig.(3): Liver of rat from paracetamol treated group.

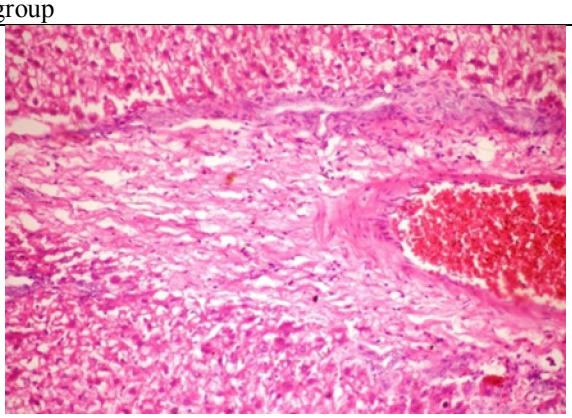

Fig (4): Liver of rat from paracetamol treated group

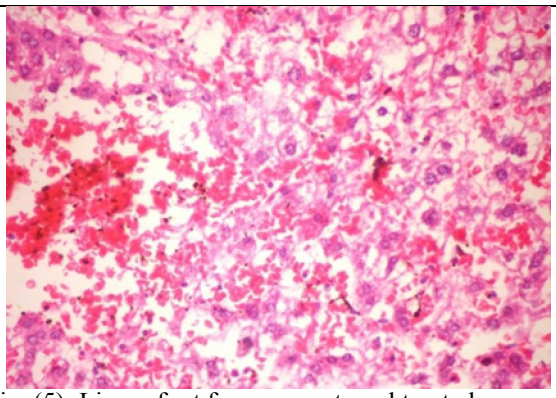

Fig. (5): Liver of rat from paracetamol treated group

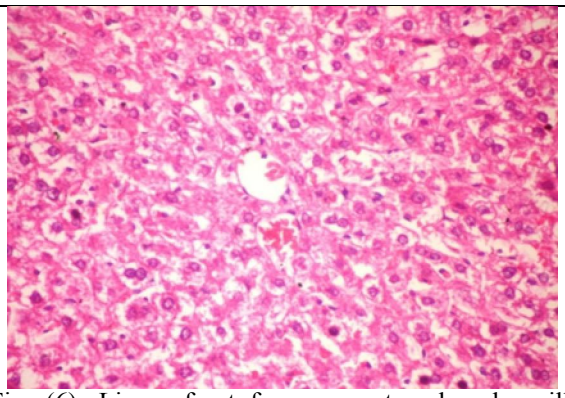

Fig. (6): Liver of rat from paracetamol and milk thistle treated group

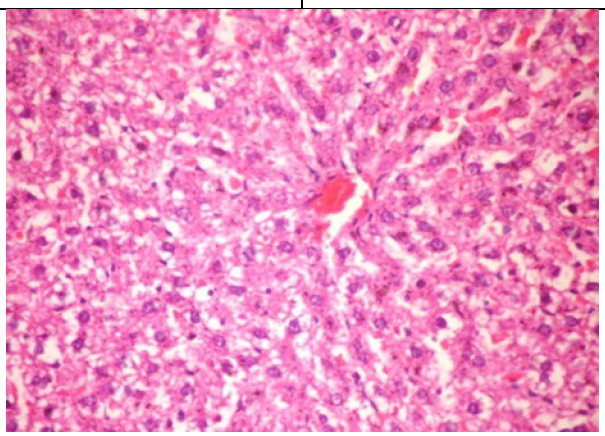

Fig. (7): Liver of rat from paracetamol and milk thistle treated group

It can be concluded that the milk thistle has a definite anti-hyperlipidemic and hepato-cardiac protective potential and substantiate its use in folk medicine and recommendations of the dietitians to use it in curing various diseases

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