*Available online*www.ijpras.com

International Journal of Pharmaceutical Research&Allied Sciences, 2019, 8(1):84-94

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Protective Impact of Curcumin against Paracetamol-Induced Hepatotoxicity in Rats

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ABSTRACT

The present study was planned to investigate the protective impact of curcumin on hepatotoxicity of paracetamol in male albino rats. Thirty-five male albino rats were allocated into seven equal groups, each of five rats, 1st group was kept as control, 2nd group orally administered tween 80 (0.5 ml/200 g b. wt) for 15 days, 3rd group received silymarin (200 mg/kg b. wt) orally for 15 days, 4th group received curcumin (200 mg/kg b. wt) orally for 15 days, 5th group orally administered paracetamol (500 mg/kg b. wt) for the last 5 days, 6th group was given silymarin and paracetamol, 7th group was given curcumin and paracetamol. The hepatotoxicity of paracetamol in rats displayed a significant reduction in erythrocytic count (RBCs), concentration of hemoglobin (Hb), the values of packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC) with a critical elevation in mean corpuscular volume (MCV), platelets (Plts), white blood cells (WBCs) and neutrophils count. Silymarin or curcumin administration with paracetamol in rats produced a significant increase in RBCs, Hb, PCV and MCHC with a significant decrease in MCV, Plts, WBCs and neutrophils count. Liver enzymes (ALT and AST) and total bilirubin were significantly increased post administration of paracetamol whereas administration of silymarin or curcumin with paracetamol significantly decreased the activities of liver enzymes and total bilirubin. Paracetamol administration in rats produced a significant increase in tumor-necrosis factor-alpha (TNF-α) level and lactate-dehydrogenase enzyme (LDH) activity. Silymarin or curcumin administration with paracetamol displayed a critical decrease in TNF-α and LDH activity. Paracetamol elicited a critical decrease in catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities with a critical elevation in malondialdehyde (MDA) concentration. Silymarin or curcumin administration with paracetamol evoked a critical elevation in CAT, SOD and GPX activities with a significant decrease in MDA concentration. Histopathological examination of paracetamol treated rats displayed alterations in liver histoarchitecture with degenerative and necrotic changes in hepatic cells but restored to nearly normal picture by pretreatment with silymarin and curcumin. Curcumin has a hepatoprotective effect on paracetamol induced-hepatotoxicity in rats.

Key words:*Silymarin, Curcumin, Paracetamol, Hepatotoxicity, Rats*

INTRODUCTION

It is an essential organ which directs numerous vital metabolic capacities and is in charge of keeping up hemeostasis of the body. It has a massive assignment of detoxification of xenobiotics, natural toxins and chemotherapeutic operators. Subsequently, this organ is subjected to assortment of infections and clutters [1].

Hepatotoxicity is a general term for liver damage, including necrosis, steatosis, fibrosis, cholestasis and vascular injury [2]. Hepatic damage by paracetamol (PCM) overdose is identified with excessive oxidative stress for the most part caused by the electrolyte and exceedingly responsive metabolite of it called N-acetyle-p-benzoquinone imine [NAPQI) [3]. Lipid peroxidation which is a secondary to NAPQI- prompted GSH depletion and oxidative stress which can cause irreversible membrane damage and cell death [4].

Silymarin is a standard extract from leafy foods of *silybum marianum*, it has been utilized for the treatment of different sicknesses in people for the most part liver related issue as it used for centuries as a hepatoprotectant [5].

Curcumin, an active principle of *curcuma longa L.* has been shown to exhibit anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, anticancer and hepatoprotective activities [6].

The purpose of this investigation is to assess the possible protective impacts of curcumin on paracetamol produced hepatotoxicity in male albino rats.

MATERIAL AND METHODS

Materials

a. Chemicals:

Paracetamol (panadol®):

Tablets (500 mg) obtained from Glaxo Smithkine, Dungarvan Ltd. Ireland and orally received in a dose of 500 mg/kg b. wt [7].

Silymarin powder (Livamarin®):

Sachets (140 mg) obtained from European Egyptian pharm. IND. Company, Alexandria, Egypt. It is orally received in a dose of 200 mg/kg b.wt. [8]

(Curcumin®):

Powder (10 g) obtained from ROTH chemical Co. (Germany), and emulsified in 2% Tween 80. Tween 80 was produced by El-Gomhoria Co., Egypt. Curcumin was orally given in a dose of 200 mg/kg b. wt[9].

b. Experimental animals

A total number of thirty-five apparently healthy adult male albino rats (weighing 200±10 g) were obtained from the Laboratory Animal House, Faculty of Veterinary Medicine, Zagazig University, Egypt. Rats were acclimatized for one week before starting of experiment. The animals were housed in metal cages under optimal conditions. They were fed on slandered diet and water *ad-libitum* during the investigation. The care and welfare of the animals confirmed to the rules of the Animal use Research Ethics Committee of Faculty of Veterinary Medicine, Zagazig University, Egypt.

Methods

1. Experimental design

Thirty-five male albino rats were allocated into seven equal groups, each of five rats. First group was kept as a control and received normal saline (1 ml/kg b.wt), 2nd group was given tween 80 in a dose of 0.5 ml/200 g b.wt per os by stomach tube once daily for 15 successive days, 3rd group was given silymarin in a dose of 200 mg/kg b.wt orally by stomach tube once daily for 15 successive days, 4th group was given curcumin in a dose of 200 mg/kg b.wt orally by stomach tube once daily for 15 successive days, 5th group was given paracetamol in a dose of 500 mg/kg b.wt orally by stomach tube once daily for the last 5 successive days, 6th group was given silymarin daily for 10 successive days and simultaneously with paracetamol for the last 5 successive days and 7th group was given curcumin daily for 10 successive days and simultaneously with paracetamol for the last 5 successive days.

2. Sampling

a. Blood samples

Animals were fasted overnight then killed under light anesthesia (Ether). Five rats from each gathering were utilized for collection of blood samples from the retro-orbital venous plexus on the 16th day of the experiment. Each blood sample was separated into 2 parts. The primary part (3 ml) was collected in clean Wassermann tubes containing disodium salt of EDTA for hematological studies. The second part (2 ml) was taken without anticoagulant in a clean centrifuge tube and centrifuged at 3000 rpm for 10 minutes to separate serum for biochemical examinations [10].

b. Tissue specimens

Liver was collected immediately after decapitation of animals under light anesthesia then washed with ordinary physiological saline and divided into two parts. The first one for antioxidant/oxidant status assessment by using an electrical homogenizer. A 0.5 g of liver tissue was homogenized in 5 ml phosphate buffer [7.4 pH) and the sample was kept on ice, then centrifugation of tissue homogenates was done at 1200 rpm for 20 minutes at 4ºC. Finally, the supernatants were isolated and stored at -80ºC until further use. The second part was fixed in neutral formalin at 10% for histopathological examination.

3. Hematological studies

RBCs, Hb, WBCs, (MCV), MCHC [11] PCV [12], and Differential leukocytic counts were determine [13].

4. Biochemical analysis

The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined [14] besides alkaline phosphatase activity (ALP) [15]. Serum total bilirubin was determined [16]. Serum tumor-necrosis factor alpha (TNF-α) was done by ELISA [17] and serum lactate dehydrogenase enzyme activity (LDH) was determined [18]

5. Antioxidant / oxidant status

Tissue catalase enzyme activity (CAT) [19], Tissue superoxidase dismutase (SOD) enzyme activity [20], Tissue glutathione peroxidase enzyme activity (GPX) [21], and Tissue malondialdehyde concentration (MDA) was determined [22, 23].

6. Histopathological examination

Specimens from liver were taken for histopathological examination and fixed in 10% neutral buffer formalin, paraffin sections of 5-micron thickness were prepared, stained with H&E stain then examined microscopically [24].

7. Statistical analysis

The obtained data were represented as mean ± SE for each gathering. The informations were dissected by one-way ANOVA followed by Duncan's test. Informations were considered significant at $P < 0.05$ [25].

RESULTS

1. Hematological results

a. Erythrogram

Tween 80, silymarin and curcumin administration produced a non-significant variation in erythrogram. Oral administration of paracetamol displayed a significant decrease in RBCs, Hb, PCV and MCHC with a significant increase in MCV and platelets count when compared with control group. Administration of silymarin or curcumin with paracetamol orally elicited a significant increase in RBCs, Hb, PCV and MCHC with a significant decrease in MCV and platelets counts when compared with paracetamol group (Table 1).

Table 1: Effect of oral administration of silymarin (200 mg/kg b. wt) or curcumin (200 mg/kg b. wt) for 15 successive days on erythrogram in normal and paracetamol (500 mg/kg b. wt for the last 5 days) induced hepatotoxicity in male albino rats (Mean ± SE) n = 5

Groups	RBCS (10^6/µl)	Hb (g/dl)	PCV (%)	MCV (Fl)	MCHC (%)	Plts (10^3/µl)
Control	7.57±0.072^{a}	16.23±0.145^{a}	52.00±1.15^{a}	68.67±0.870^{c}	31.24±0.834^{a}	349.33±8.96^{c}
Tween	7.49±0.063^{a}	16.63±0.176^{a}	51.00±1.15^{a}	68.06±2.12^{c}	32.63±0.566^{a}	349.66±10.26^{c}
Silymarin	7.58±0.053^{a}	16.43±0.202^{a}	51.00±1.52^{a}	67.20±1.54^{c}	32.30±1.35^{a}	344.33±5.23^{c}
Curcumin	7.52±0.114^{a}	16.53±0.120^{a}	50.67±2.33^{a}	67.45±4.04^{c}	31.79±0.806^{a}	342.33±7.88^{c}
Paracetamol	3.34±0.094^{d}	9.00±0.173^{d}	37.66±1.45^{b}	112.83±1.81^{a}	23.94±0.728^{b}	456.33±15.01^{a}
Silymarin+paracetamol	5.92±0.135^{b}	14.40±0.321^{b}	49.00±1.15^{a}	82.77±0.895^{bc}	29.38±0.110^{a}	375.33±11.09^{b}
Curcumin+paracetamol	4.96±0.270^{c}	11.63±0.120^{c}	46.33±0.881^{a}	93.95±5.33^{b}	27.12±0.443^{a}	388.66±11.56^{b}

Means within the same column in each category carrying different superscript letters are significant at $P < 0.05$

b. Leukogram

Tween 80, silymarin and curcumin administration produced a non-significant variation in leukogram. The oral receiving of paracetamol in rats revealed a critical elevation in WBCs count beside neutrophil count comparing with control group. Oral administration of silymarin or curcumin with paracetamol produced a significant decrease in WBCs and neutrophil count when compared with paracetamol group (Table 2).

2. Serum biochemical results

a. Liver enzyme activities and total bilirubin

Non-significant variations were recorded in liver enzymes post administration of tween 80, silymarin and curcumin. The present study reported that oral administration of paracetamol in rats produced a significant increase in ALT and AST activities and total bilirubin level comparing with control group. Oral administration of silymarin or curcumin with paracetamol elicited a critical reduction in ALT and AST activities and bilirubin

level when compared with paracetamol group (Table 3).

b. Tumor necrotic factor-alpha (TNF-α) and lactate dehydrogenase enzyme activity (LDH)

Non-significant changes were recorded in TNF-α and LDH post administration of tween 80, silymarin and curcumin. Oral receiving of paracetamol in rats evoked a critical increase in TNF-α level and LDH activity comparing with control group. Administration of silymarin or curcumin with paracetamol in rats resulted in a critical reduction in TNF-α level and LDH activity when compared with paracetamol group (Table 4).

Table 2: Effect of oral administration of silymarin (200 mg/kg b. wt) or curcumin (200 mg/kg b. wt) for 15 successive days on leukogram in normal and paracetamol (500 mg/kg b. wt for the last 5 days) induced hepatotoxicity in male albino rats (Mean ± SE) n = 5

Groups	WBCS (10^3/µl)	Differential leukocytic count			
		Lymphocytes (10^3/µl)	Neutrophil (10^3/µl)	Eosinophil (10^3/µl)	Monocytes (10^3/µl)
Control	8.89±0.098^{c}	6.26±0.066^{a}	1.30±0.035^{d}	0.53±0.046^{a}	0.79±0.023^{a}
Tween	8.88±0.066^{c}	6.25±0.070^{a}	1.31±0.038^{d}	0.51±0.025^{a}	0.80±0.041^{a}
Silymarin	8.87±0.077^{c}	6.27±0.098^{a}	1.27±0.032^{d}	0.55±0.028^{a}	0.77±0.068^{a}
Curcumin	8.83±0.120^{c}	6.23±0.058^{a}	1.29±0.011^{d}	0.53±0.011^{a}	0.79±0.050^{a}
Paracetamol	11.48±0.265^{a}	6.17±0.126^{a}	3.94±0.299^{a}	0.57±0.046^{a}	0.79±0.066^{a}
Silymarin+paracetamol	9.50±0.174^{b}	6.22±0.057^{a}	1.91±0.158^{c}	0.57±0.035^{a}	0.79±0.060^{a}
Curcumin+paractamol	9.79±0.179^{b}	6.19±0.032^{a}	2.31±0.156^{b}	0.56±0.026^{a}	0.73±0.039^{a}

Means within the same column in each category carrying different superscript letters are significant at P < 0.05

Table 3: Effect of oral administration of silymarin (200 mg/kg b. wt) or curcumin (200 mg/kg b. wt) for 15 successive days on liver enzyme activities and total bilirubin in normal and paracetamol (500 mg/kg b. wt for the last 5 days) induced hepatotoxicity in male albino rats (Mean ± SE) n = 5

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (mg/dl)
Control	11.66±0.881^{d}	27.00±2.30^{c}	83.00±5.68^{a}	1.29±0.069^{c}
Tween	11.33±1.76^{d}	27.00±1.52^{c}	86.33±2.33^{a}	1.17±0.129^{c}
Silymarin	11.33±0.881^{d}	26.33±1.20^{c}	83.00±1.15^{a}	1.30±0.008^{c}
Curcumin	11.00±1.52^{d}	25.66±4.70^{c}	86.66±3.75^{a}	1.22±0.028^{c}
Paracetamol	83.33±4.25^{a}	65.66±2.40^{a}	88.00±1.15^{a}	2.20±0.035^{a}
Silymarin+paracetamol	36.00±3.05^{c}	25.33±4.84^{c}	83.33±6.38^{a}	1.25±0.056^{c}
Curcumin+paracetamol	50.00±3.78^{b}	40.33±1.45^{b}	84.33±7.21^{a}	1.43±0.058^{b}

Means within the same column in each category carrying different superscript letters are significant at P < 0.05

Table 4: Effect of oral administration of silymarin (200 mg/kg b. wt) or curcumin (200 mg/kg b. wt) for 15 successive days on serum tumor necrotic factor alpha (TNF-α) and lactate dehydrogenase (LDH) in normal and paracetamol (500 mg/kg b. wt for the last 5 days) induced hepatotoxicity in male albino rats (Mean ± SE) n = 5

Groups	TNF-α (pg/ml)	LDH (U/L)
Control	49.52±2.06^{d}	165.09±3.20^{d}
Tween	50.57±1.47^{d}	167.46±2.55^{d}
Silymarin	50.59±0.70^{d}	168.15±4.53^{d}
Curcumin	50.86±1.22^{d}	166.54±5.47^{d}
Paracetamol	120.38±4.70^{a}	257.63±3.99^{a}
Silymarin + paracetamol	69.30±0.91^{c}	188.47±1.27^{c}
Curcumin + paracetamol	82.89±3.11^{b}	209.04±2.77^{b}

Means within the same column in each category carrying different superscript letters are significant at P < 0.05

3. Hepatic antioxidant/oxidant status

Administration of tween 80, silymarin and curcumin produced non-significant changes in hepatic antioxidant and oxidant status.

The present examination indicated that oral administration of paracetamol in rats produced a critical decline in catalase (CAT), superoxidase dismutase (SOD) and glutathione peroxidase (GPX) activities with a critical increase in malondialdehyde (MDA) concentration when compared with control group.

Silymarin or curcumin administration with paracetamol in rats displayed a critical increase in CAT, SOD and GPX activities with a critical reduction in MDA concentration when compared with paracetamol group (Table 5).

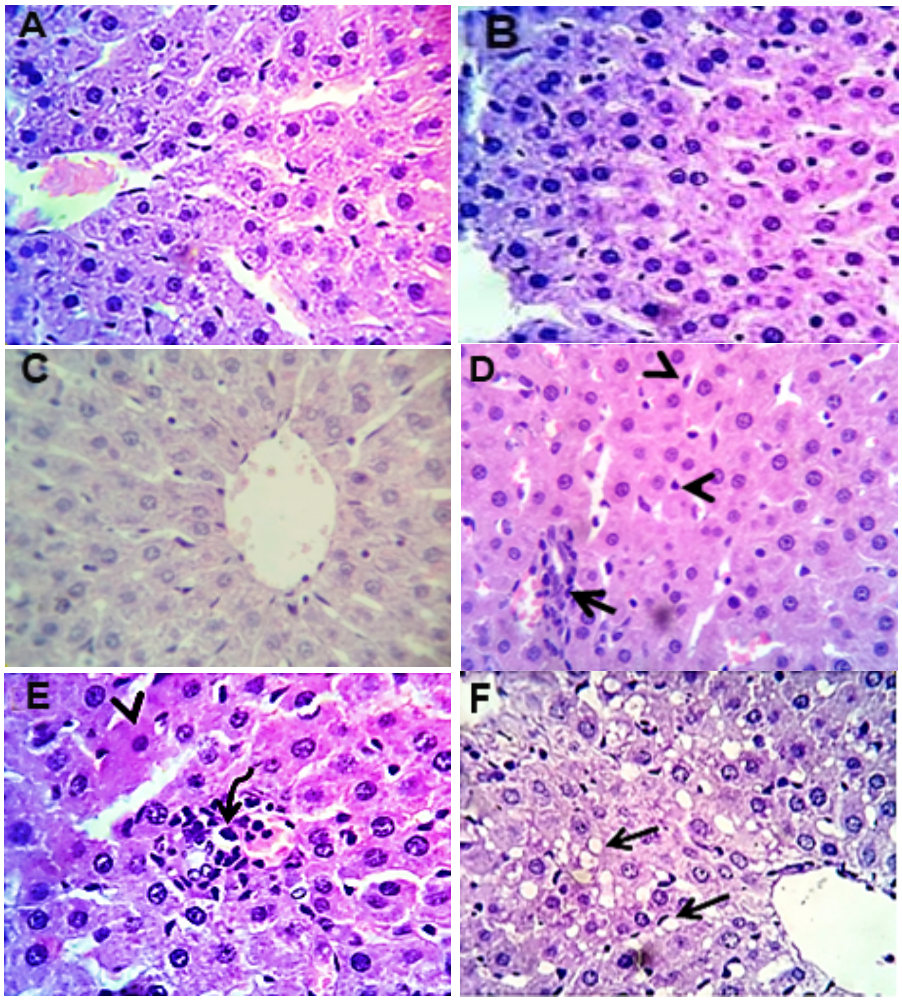
4. Liver histopathology

Control, tween 80, silymarin and curcumin groups showed normal hepatic histomorphological structures but in paracetamol treated group there was alterations in liver architecture, there were also focal necrosis and individual apoptosis of hepatocytes. All of these changes were mildly improved by silymarin and curcumin treated as shown in plate (1).

Table 5: Effect of oral administration of silymarin (200 mg/kg b. wt) or curcumin (200 mg/kg b. wt) for 15 successive days on hepatic antioxidant and oxidant status in normal and paracetamol (500 mg/kg b. wt for the last 5 successive days) induced hepatotoxicity in male albino rats (Mean ± SE) n = 5

Groups	CAT (µ/g)	SOD (µ/g)	GPX (µ/g)	MDA (nmol/g)
Control	0.82±0.081^{a}	2.60±0.125^{a}	114.46±2.41^{a}	5.01±0.195^{c}
Tween 80	0.85±0.037^{a}	2.44±0.342^{a}	113.68±1.49^{a}	4.94±0.026^{c}
Silymarin	0.85±0.055^{a}	2.36±0.317^{a}	115.46±2.96^{a}	5.03±0.110^{c}
Curcumin	0.88±0.026^{a}	2.31±0.135^{a}	114.21±1.94^{a}	4.95±0.104^{c}
Paracetamol	0.24±0.032^{d}	0.55±0.031^{b}	51.84±2.54^{d}	22.30±1.89^{a}
Silymarin+paracetamol	0.62±0.044^{b}	1.72±0.146^{a}	97.95±1.52^{b}	7.45±0.407^{b}
Curcumin+paracetamol	0.44±0.029^{c}	1.35±0.037^{a}	81.10±3.48^{c}	9.58±0.345^{b}

Means within the same column in each category carrying different superscript letters are significant at P < 0.05.



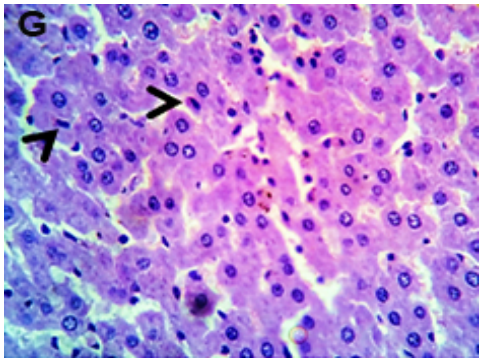


Plate 1. Photomicrographs of rat liver sections in different experimental groups stained with H&E x 400.

A, B, C and D: Liver sections in control, tween 80, silymarin and curcumin groups respectively exhibited normal hepatic histomorhological structures with hypertrophied Kupffer's cells (arrowheads) and a few inflammatory cells were seen in portal area (arrow) in group D (curcumin group). E: Rat liver section in paracetamol treated group revealed mild biliary proliferation, focal necrosis, individual cells apoptosis (arrowhead) and the hepatic sinusoids showed lymphocytosis (curved arrow). F: Liver section in silymarin and paracetamol treated group showed apparently normal hepatic parenchyma with mild to moderate centrilobular vacuolation (fatty changes) (arrows). G: Liver section in curcumin and paracetamol treated rats showed hypertrophied Kupffer's cells (arrowheads). Generally, there was an improvement in silymarin + paracetamol group and curcumin + paracetamol group.

DISCUSSION

The liver is the real organ responsible for digestion; detoxification and secretory function in the body. It is particularly susceptible to chemically-induced injury, so it is the most important target for toxicity caused by drugs [26].

Hepatotoxicity is associated with impaired liver functions caused by exposure to drug or another non-infectious agent, manifested by elevation in liver enzymes [27].

Curcumin has strong antioxidant, anti-inflammatory and other activities. Likewise, it is well tolerated at a very high dose with not toxic impacts. So, it has the potential for the development of modern medicine for the treatment of various diseases [28].

In view of these information, the present investigation aims to trace the antioxidant and hepatoprotective impacts of curcumin on paracetamol induced hepatotoxicity in male albino rats.

In this study, paracetamol administration induced a critical decrease in RBCs, Hb, PCV and MCHC with critical elevation in MCV, Plts, WBCs and neutrophil counts.

Similar findings were reported by [29] who recorded a significant decrease in RBCs, Hb and PCV with a critical elevation in WBCs count in rats medicated with paracetamol (300 mg/kg b. wt intraperitoneal for 2 days). The previous authors mentioned that a significant increase in total leukocytic count could be as a result of the body defense mechanism trying to protect the body from being vulnerable to infections following liver damage. The decrease in RBCs count may be attributed to the reduction in erythropoiesis in bone marrow and faster rate of destruction of peripheral RBCs in spleen [10]. The reduced Hb content may be due to reduction in size of RBCs, impaired biosynthesis of haem in bone marrow or because of decrease in the rate of formation of RBCs. Paracetamol also cause significant decrease in PCV value and that indicated the induction of anaemia.

The present results of current study are similar to [30] who recorded that rats treated with 7.5 mg paracetamol/kg b. wt for 42 days showed non-significant changes in lymphocytes, eosinophils, monocytes and basophils counts.

The obtained results disagreed with [31] who observed a reduction in total leukocytic count, lymphocytic count and polymorphnuclear cells of Wister rats administered with paracetamol at 2 g/kg b. wt once daily for 14 days. The difference in leukogram may be due to the difference in dose, route of administration, duration and animal species.

The present results suggested that administration of silymarin or curcumin prior and simultaneously with paracetamol showed an improvement in erythrogram values (RBCs, Hb, PCV, MCHC) with a significant

decrease in MCV and Plts count as compared to paracetamol group. Similar findings were reported [32] who stated that curcumin administration to infected mice improved erythrocytic count, Hb and blood indices.

Hepatic cells contain a gathering of enzymes which have been utilized as markers for observing liver damage. The enzymes alanine amino-transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels are increased following liver cell damage [33].

The current study revealed that paracetamol overdose is evident with increasing of serum transaminases and bilirubin levels compared with control group.

Such recorded results are in agreement with previous reports [34, 35] who reported that rats received overdose of paracetamol showed higher levels of ALT, AST and bilirubin. The present data agreed with [36] who found that paracetamol significantly increased ALT, AST and bilirubin levels in mice. Furthermore [37] said that acute paracetamol toxicity resulted in remarkable elevation in the activities of plasma ALT, AST and ALP.

Abnormal state of AST demonstrates liver harm, for example, that caused by viral hepatitis and cardiac infarction and muscle damage. AST catalyzes the change of alanine to pyruvate and glutamate and is discharged in a similar manner. In this manner, ALT is more particular to the liver and is a superior parameter for identifying liver damage. The raised activities of hepatic enzymes are demonstrative for cellular leakage and loss of functional integrity of cell membrane in liver [1,38].

In the present study, hyperbilirubinemia recorded in paracetamol treated group might be as a result of improper uptake, metabolism and discharge of bile by the diseased hepatic cells [39]. These findings are confirmed by [40] who reported that centri-zonal and focal necrosis of liver with hepatocytes ballooning in the liver of albino mice intoxicated by paracetamol (500 mg/kg b. wt orally). The present study indicated that administration of silymarin prior and simultaneously with paracetamol improved hepatic enzymes and total bilirubin. The liver protective mechanism of silymarin may be related to its stimulation of RNA polymerase enzyme and protein synthesis which is vital role in the repair of hepatic damage and it's necessary for restoring structural proteins and enzymes damaged by toxins [41] The same was noticed by [8] who reported that rat received 200 mg/kg b. wt of silymarin for 7 days after paracetamol intoxication showed a critical reduction in the activities of ALT, AST and ALP and restored control value. Post treatment of silymarin (100 mg/kg b. wt) for 7 days after APAP administration caused critical reduction in ALT and AST levels in serum [35].

The current study revealed that administration of curcumin prior and simultaneously with paracetamol elicited a critical reduction in serum bilirubin and liver enzymes levels.

These results are in harmony with [42] who found that receiving of single dose of curcumin (400 mg/kg orally) with paracetamol dose decline the activities of transaminases, ALP and total bilirubin level.

In this study, paracetamol administration showed a significant elevation in serum TNF-α and LDH. Similar findings were achieved by [7] who stated that oral administration of paracetamol (500 mg/kg) for the last 5 progressive days of experiment to rats revealed a marked increase in serum TNF-α and LDH compared to control one. The obtained results coordinates [43] who observed that lactate dehydrogenase enzyme (LDH) in paracetamol medicated rats was critically elevated throughout 21 days of experiment. In the same manner [44] noticed that single intraperitoneal injection of paracetamol (750 mg/kg b. wt) in rats. The first day of experiment, revealed a critical increase in LDH activity. The increase in cystolic LDH activity by paracetamol may be because of the intracellular collection of Ca^{2+}, which results in initiation of phosphofructo-kinase and anaerobic glycolysis prompting lactate formation [45]. Loss of Ca^{2+} hemeostasis because of oxidative damage and increment in intracellular Ca^{2+} has been reported to a late and may be irreversible final stage during the process of cell death for paracetamol [46].

The present results are in harmony with [47] who reported that paracetamol induced toxic damage to rat hepatocytes as assessed by critical increase in LDH leakage.

The recorded results suggested that administration of silymarin or curcumin prior and simultaneously with paracetamol resulted in a critical decline in TNF-α and LDH.

The obtained results go hand in hand with those recorded by [7] who found that oral administration of curcumin alone for 10 days and with the paracetamol for the last 5 days restored the TNF-α and LDH values to normal. The previous authors revealed that curcumin administration controlled serum and hepatic LDH. Notwithstanding, it didn't standardize the LDH level totally as it stayed lesser than paracetamol injected rats.

In this examination, paracetamol administration in the last 5 progressive days induced oxidative stress in rat livers, as prove by high critical decrease in antioxidant enzyme activities (CAT, SOD and GPX) and a critical elevation in MDA generation.

Lipid peroxidation influences the liver to more prominent degree causing the formation of high molecular mass protein aggregated with the membrane. Thus, the expansion in the level of MDA is a pointer of lipid peroxidation [48]. From our opinion, overproduction of free radicals and reactive oxygen species during paracetamol metabolism leads to exhaustion of natural body antioxidant system and enhanced lipid peroxidation as evidenced by the observed decrement in the level of catalase, SOD and GPX and the increase of MDA concentration in the liver homogenate.

The reactive oxidative stress attacks polyunsaturated fatty acids and disturbs the cell membrane. It prompts oxidative lipid and form MDA, a product of lipid peroxidation. The Increase in production of liver MDA reported in our trials by paracetamol are coincided with previous study which reported that paracetamol increased extracellular MDA level [49].

The recorded results are in agreement with works of [50] who observed that administration of paracetamol at 2 g/kg for 7 days caused a critical elevation in MDA level.

Similar results were noticed by [51] who reported that rats received 500 mg/kg b. wt. of paracetamol for 7 days exhibited a critical elevation in MDA level.

The present results suggested that administration of curcumin or silymarin prior and simultaneously with paracetamol resulted in a critical elevation in the activities of antioxidant enzymes (CAT, SOD and GPX) and a critical reduction in activity of MDA compared with paracetamol group.

SOD is an antioxidant enzyme that changes superoxide anion $O_2^{\cdot}$ to H_2O_2. CAT converts H_2O_2 to water and O_2. GPX catalyzes the decreasing of H_2O_2 and other peroxides by coupling reduced glutathione [52, 53].

The recorded data are in line with [7] who mentioned that hepatic lipid peroxidation level was inhibited by receiving of curcumin to paracetamol-treated rats.

The obtained results are in harmony with [51] who reported that paracetamol-treated rats received curcumin at a dose of 200 mg/kg b. wt for 7 days revealed an increase in the levels of antioxidant enzymes and decreasing the lipid peroxidation.

The present investigation reported that administration of silymarin prior and simultaneously with paracetamol resulted in restoring the antioxidant enzyme activities to control and decline the MDA concentration level. The antioxidant activity of silymarin to the free hydroxyl groups present in its structure that may favor the decline of lipid peroxidation by reacting with peroxy radicals, thus leading to rise in the cellular antioxidant defense mechanism [54]. These results agree with [55] who observed that treatment of paracetamol intoxicated rats with silymarin (100 mg/kg orally) for 10 days caused a critical reduction in the hepatic MDA level and enhanced the antioxidant enzymes (CAT, SOD and GPX).

In the present study, the microscopical examination of liver sections of rats induced hepatotoxicity with paracetamol revealed a moderate congestion of the hepatic blood vessels, degenerative and necrotic changes in a mild to moderate number of hepatic cells (25-30%), portal aggregation of round cells and mild biliary proliferation, in addition to focal individual cells necrosis and apoptosis with replacement of dead cells with round cells could be detected. On agreement [56] who noticed that liver of paracetamol treated rats (600 mg/kg single intraperitoneal injection) exhibited vacuolization of hepatocytes, sinusoidal dilation, infiltration of Kupffer cells and fatty degeneration with respect to normal control one. Moreover, paracetamol treated group showed a high derangement of hepatic cords, ballooning necrosis, cellular infiltration and loss of cell boundaries without any signs of regeneration [57].

CONCLUSION

Our observations recommended that curcumin improved the harmful impacts of paracetamol-induced hepatotoxicity in male albino rats. The defensive part of curcumin against paracetamol-initiated damages might result from its antioxidative and anti-inflammatory impacts.

REFERENCES

1. Rajkapoor B, Venugopal Y, Anbu J, Harikrishman N, Gobinath M, Ravichandran V. Protective effect of phyllanthus polyphllus on acetaminophen induced hepatotoxicity in rats. Pak. J. Pharm. Sci. 2008; 21 (1): 90-93.
2. Ishak KG, Zimmerman HJ. Morphologic spectrums of drug- induced liver disease. Gastroenterol. Clin. North. Am., 1995; 24 (4): 759-786
3. Michael SL, Pumford NR, Mayeux PR, Niesman MR, Hinson JA. Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species, Hepatology 1999; 30:186-195.
4. Kyle ME, Miccadei S, Nakac D, Farber JL. Superoxide dismutase and catalase protect cultured hepatocytes from the cytotoxicity of acetaminophen. Biochem. Biophys. Res. Gommun., 1987; 149: 889-894.
5. Saller R, Meier R, Brignoli R. The use of silymarin in the treatment of liver diseases. Drugs 2001; 61: 2035-2063.
6. Folwarczna J, Zych M, Trzeciak HI. Effects of curcumin on the skeletal system in rats. Pharmacological Reports 2010; 62 (5): 900-909.
7. Farghaly HS and Hussein MA. Protective effect of curcumin against paracetamol-induced liver damage. Aust. J. Basic Appl. Sci., 2010; 4 (9): 4266-4274.
8. Kanchanai N, Sadlq AM. Hepatoprotective effect of Plumbago Zeylanica on paracetamol induced liver toxicity in rats. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(1): 151-154.
9. Singh R, Sharma P. Hepatoprotective effect of curcumin on Lindane-induced oxidative stress in male Wistar rats. Toxicol. Int., 2011; 18 (2): 124-129.
10. Coles EH. Veterinary Clinical Pathology. 4th ed., WB. Saunders Company, Philadelphia, London, Toronto, Mexico, Sydney, Tokyo, Hong Kong 1986.
11. Jain NC. Schalm's Veterinary Heamatology. 4th Ed., Lea and Fibiger, Philadelphia, USA. 1986.
12. Decie JV, Lewis SM. Practical Haematology. 7th edition, ELBS with Churchill Livingston, England, 1991; 37-85.
13. Mitruka BM, Rawnsley H. Clinical, biochemical and haematological references values in normal experimental animals. Mason publishing USA Inc., 1977; 53-54.
14. Reitiman S, Frankel S. Colorimetric method for determination of serum transaminases activities. Am. J. Clin. Path., 1957; 28:56-68.
15. Moss DW. Alkaline Phosphatase isoenzymes. Clin. Chem., 1982; 28 (10): 2007-2016.
16. Doumas BT, Perry BW, Sasse EA. Standardization in bilirubin assays: Evolution of selected method and stability of bilirubin solution. Clin. Chem., 1973; 19: 984-993.
17. Corti A, Fassino J, Marcucci F, Barbenti E, Cassani G. Oligometric tumor necrosis factor-α slowly converts into the reactive forms at bioactive levels. Biochem. J., 1992: 284: 905-910.
18. Siekmann L, Bonora R, Burtis CA, Ceriotti F, Clero-Renaud P, Ferard G. IFCC primary reference procedures for the measurement of catalytic activity concentrations of catenzymes at 37 degrees C. Part 3. Reference procedure for the measurements of catalytic entration of lactate dehydrogenase. Clin Chem Lab Med. 2002; 40 (6): 643-648.
19. Aebi H. Catalase in-vitro. In: Packer L, editor. Methods of Enzymology. Vol. 105. San Diego: Academic Press Incpp. 1984; 121–126.
20. Nishikimi M Roa NA, Yogi K. Measurement of superoxide dismutase. Biochpa.Biochem. Bioph. Res. Common., 1972; 46:849-854.
21. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 1967; 70 (1): 158-169.
22. Satoh K. Serum lipid peroxide in cerebrovascular disorders O^ determined by new colorimeteric method. Clin. Chimica. Acta., 1978; 37-43.
23. Ohkawa H, Oshishi N, Yagi K. Assay for lipid peroxidase in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 1979; 95 (2): 351-358.
24. Bancroft JD, Gamble M. Theories and Practical of Histopathological Techniques. 6th Ed., Chirchill Livingstone, New York, London, Madrid, 2013.

25. Tamhane AC, Dunlop DD. Statistics and data analysis from elementary to intermediate. Upper Saddle River, USA. 2000.
26. Kamel HH, Abd El-Rahman AH, Ahmed MSW, Mohamed AH. Protective effect of some antioxidants against CCL4-induced toxicity in liver cells from BRL3A cell line. Journal American Science 2010; 6 (10): 992-1001.
27. Victor J, Navarro MD, Senior JR. Drug-related hepatotoxicity. The New England Journal of Medicine 2006; 54: 731-739.
28. Nawaz A, Khan GM, Hussain A, Ahmad A, Khan A, Safdar M. Curcumin: A natural product of biological importance. Gomal University Journal of Research 2011; 27 (1): 07-14.
29. Samuel SA, Francis AO, Ayomide O, Onyinyechi UO. Effects of paracetamol-induced liver damage on some hematological parameters: Red blood cell count, White blood cell count (RBC) count, (WBC) count and packed cell volume (PCV) in wistar rats of either sex. Indo American Journal of Pharmaceutical Research 2015; 5: 2593-2599.
30. Oyedeji KO, Bolarinwa AF, Ojeniran SS. Effect of paracetamol on haematological and reproductive parameters in male albino rats Journal of Pharmacy and Niological Science, 2013; 4 (6): 2278-3008.
31. Senthilkumar R, Chandran R, Parimelazhagan T. Hepatoprotective effect of Rhodiola imbricate rhizome against paracetamol-induced liver toxicity in rats. Saudi Journal of Biological Sciences, 2014; 21: 409-416.
32. Sharma B, Sharma C, Sharma C. Influence of curcuma longa and curcumin on blood profile in mice subjected to aflatoxin B1. International Journal ofPharmaceutical Sciences and Research 2011; 2 (7): 1740-1745.
33. Hukkeri VI, Jaiprakash B, Lavhale MS, Karadi RV, Kuppast IJ. Hepatoprotective activity of Anthus excels Rpxn. Leaf extrcts on experimental liver damage in rats. J. Pharmacogny, 2002; 11: 120-128.
34. Olaleye MT, Akinmoladun AC, Ogunboye AA, Akindahunsi AA. Antioxidant activity and hepatoprotective property of leaf extracts of Boerhaavia diffusa Linn against acetaminophen- induced liver damage in rats. Food and Chemical Toxicology 2010; 48: 2200-2205.
35. Bektur NE, Sahin E, Baycu C, Unver G. Protective effects of silymarin against acetaminophen-induced hepatotoxicity and nephrotoxicity in mice. Toxicology and Industrial Health 2016; 32 (4): 589-600.
36. Nithianantham K, Shyamala M, Chen Y, Latha LY, Jothy SL, Sasidharan S. Hepatoprotective potential of clitoria ternatea leaf extract against paracetamol induced damage in mice. Molecules 2011; 16 (12): 10134.
37. Abdel-Azeem AS, Hegazy AM, Ibrahim KS, Farrag AR, El-Sayed EM. Hepatoprotective, antioxidant and ameliorative effects of ginger (Zingiber officinale Roscoe) and vitamin E in acetaminophen treated rats. J. Diet. Suppl., 2013; 10 (3): 195-209.
38. Drotman R, Lawhan G. Serum enzymes are indications of chemical induced liver damage. Drug and Chemical Toxicology, 1978: 1: 163-171.
39. Wolf A, Diez-Fernandez C, Trendelenburg CF, Prieto P, Hary S, Trammer WE. Paracetamol induced oxidative stress in rat hepatocytes. J. Pharm. Exp. Ther., 1997; 280: 1328-1334.
40. Tabassam N, Agrawal SS. Hepatoprotective activity of eclipta Alba Hassk against paracetamol induced hepatocellular damage in mice. JK-Practitioner. 2004; 11 (4): 278- 280.
41. Tyutyulkova N, Gorantcheva U, Tuneva S. Effect of silymarin (carsil) on the microsomal glycoprotein and protein biosynthesis in liver of rats with experimental galactosamine hepatitis. Methods Find Exp. Clin. Pharmacol., 1983; 5: 181-184.
42. Reham E. Masoud (2017): Hepatoprotective effect of curcumin versus silymarin on paracetamol induced hepatotoxicity in rats. Int J Pharm Bio Sci., 8 (2): 134-141.
43. Lebda MA, Taha NM, Korshom MA, Mandour AEA. Ginger (Zingiber officinale) potentiate paracetamol induced chronic hepatotoxicity in Rats. J. Med. Plant Res., 2013; 7 (42):3164–3170.
44. Ozougwu JC, Elom MO, Obimba KC, Obiukwu CE, Usanga VU. Ameliorative effects of Zingiber officinale extracts against experimentally-induced hepatotoxicity in rats. Am. Euras. J. Toxicol. Sci., 2016; 8 (2): 69-76.
45. Landowne D, Ritchie JM. On the control of glycogenolysis in mammalian nervous tissue by calcium. J. Physiol., 1971; 212: 503-517.

46. Strubelt O, Younes M. The toxicological relevance of paracetamol-induced inhibition of hepatic respiration and ATP depletion. Biochem. Pharmacol., 1992; 44: 163-170.
47. Lotkova H, Kučera O, Roušar T, Endlicher R, Křiváková P, Garnol T, Červinková Z. Effect of S-adenosyl methionine on acetaminophen-induced toxic injury of rat hepatocytes in vitro acta. Vet. Brno., 2009; 78: 603-613.
48. Pryor WA. Free radical reactions and their importance in biochemical systems. Fed. Proc. Fed. Amer. Soc. Exp. Biol., 1973; 69:32
49. Boonruamkaew P, Chonpathompikunlert P, Nagasaki Y. REDOX nanoparticle therapeutics for acetaminophen-induced hepatotoxicity in mice. Oxid. Med. Cell. Longev., 2016; 1-10.
50. Yanpallewar SU, Sen S, Tapas S, Kumar M, Raju SS, Acharya SB (2002): Effect of Azadirachta indica on paracetamol-induced hepatic damage in albino rats. Phytomedicine, 9: 391-396.
51. Tung BT, Hai NT, Son PK. Hepatoprotective effect of Phytosome Curcumin against paracetamol-induced liver toxicity in mice. Braz. J. Pharm. Sci., 2017; 53 (1): 16136-16149.
52. Madrigal-Santillan E, Madrigal-Bujaidar E, Alvarez-Gonzalez I, Sumaya-Martinez MT, Gutierrez-Salinas J, Bautista M, Morales-Gonzalez A, Garcia-Luna M, Gonzalez-Rubio Y, Aguilar-Faisal JL, Morales-Gonzalez JA. Review of natural products with hepatoprotective effects. World J. Gastroenterol., 2014; 20 (40): 14787-14804.
53. Thanh TB, Thanh HN, Minh HPT, Le-Thi-Thu H, Ly HDT, Duc LV. Protective effect of Tetracera scandens L. leaf extract against CCl4-induced acute liver injury in rats. Asian Pacific J. Trop. Biomed., 2015; 5 (3): 221-227.
54. Ramadan L, Roushdy HM, Abu Senna GM, Amin NE, El-Deshw OA. Radioprotective effect of silymarin against radiation induced hepatotoxicity. Pharmacol. Res., 2002; 45: 447-452.
55. Kumar MR, Phaneendra P, Bodhanapu S, Fasalu ROM, Mohamed OK, Tamizmani T. Antioxidant and hepatoprotective activity of the aqueous extract of Myrtus Communis (Myrtle) Linn. Leaves. Pharmacologyonline,2011; 1: 1083-1090.
56. Abdel-Azeem AS, Amany MH, Khadiga SI, Farrag AH, Eman ME. Hepatoprotective, antioxidant and ameliotative effects of gingers (Zingiber officinale Roscoe) and vitamin E in acetaminophen treated rats. J. Dietary Suplements, 2013; 10 (3): 195-209.
57. Kolakota R, Santosh Kumar R, Pantnaik SK. In vitro antioxidant activity and hepatoprotective potential of Ceropegia spiralis against paracetamol induced liver injury. J. Appl. Pharma. Sci., 2017; 7 (9): 199-206.