Hepatoprotective activity of Artichoke *(Cynara scolymus)* against Paracetamol toxicity in female rats

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Abstract

Hepatotoxicity is an acute adverse effect of paracetamol overdose which could be fatal, so in this research we studied the effect of paracetamol on liver enzymes (GPT, GOT, total protein, glucose, bilirubin) of female rats that drenched Artichoke. twenty laboratory female rats housed in plastic cages in animal house at university Karbala of / college of pharmacy , the animals divided randomly into four groups ,(G1 control, G2 drenched with 500 mg/kg Artichoke and 500 mg/kg paracetamol, G3 drenched with 500 mg/kg paracetamol ,G4 drenched with 500 mg/kg Artichoke) , the present study found the ability of Artichoke to protect liver enzyme against the poisonous effect of paracetamol by reducing the higher value of GPT,GOT, glucose, and evaluated the lower value of bilirubin and total protein causing by paracetamol. Histopathological changes in liver were greatly reduced on animals treated with artichoke and paracetamol when compared with the paracetamol group .However, animals treated with paracetamol showed severe congestion in central vein and there is inflammatory cell and degeneration in hepatocyte enlargement in sensoides and necrosis and odema.

Keywords: artichoke , paracetamol , liver enzymes.

الخلاصة:

ان حالات تسمم الكبد الناتج من الجرع الزائدة من البراسيتامول ممكن ان تكون مميتة,لذلك في هذا البحث درسنا تاثير البراسيتامول على انزيمات الكبد (GPT, GOT, total protein, glucose, bilirubin) لاناث الجرذان المجرعة للخرشوف .حيث استخدمنا عشرون جرذا مختبريا من البيت الحيواني في كلية الصيدلة/جامعة كربلاء وقسمت الحيوانات الى اربعة مجاميع (مج1 السيطرة ,مج2 500 ملغم/كغم خرشوف +500 ملغم/كغم براسيتامول ,مج3 500 ملغم/كغم براسيتامول ,مج4 500 ملغم/كغم خرشوف). حيث ان البحث درس قابلية ارتيجوك لحماية الكبد من التاثير السمي للبراسيتامول من خلال تقليل الارتفاع الحاصل في انزيمات الكبد من جراء التاثير السمي للكبد .ووجد تاثير معنوي كبير لهذا النبات في الحد من سمية البراسيتامول .

المفتاح: البراسيتامول ,انزيمات الكبد ,الخرشوف

1. Introduction:

Paracetamol , also called acetaminophen (N-acetyl-p-aminophenol) also was discovered in Germany at the end of the 19th century, but was not widely used until mid way through the 20th century(1). Paracetamol is a widely used analgesic and antipyretic,produces acute liver damage if overdoses are consumed .In spite of paracetamol is mainly metabolized in the liver by conjugation with glucuronide and sulphate (2,3). The hepatotoxicity paracetamol has been attributed to the formation of toxic metabolite when apart of Paracetamol is metabolized by hepatic cytochrome P-450 (4) to a highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI) (5). NAPQI is

initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid [6], but when the rate of NAPQI formation exeeds the rate of detoxification of GSH,it oxidizes tissue macromolecules such as lipid or –SH group of protein and alters the homeostasis of calcium after depleting GSH [7]. Paracetamol overdose, either alone or in combination with other drugs, is account for 60% of acute liver failure and leading to orthotropic liver transplant in the United States of America and United Kingdom [8]. Liver is an important organ in human body and the chief site for regulating internal chemical environment, liver injury induced by hepatotoxins has been recognized as a majors toxicological problem for years[9]. Also liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health, but there is not much drug available for the treatment of liver disorders [10]. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease [11]. These natural compounds that containing antiradical constituents are gaining importance in prevention and treatment of oxidative stress linked-disease [12]. Artichoke (Cynara scolymus L.) is a member of the Asteraceae family. Ezz El-Din *et al*. (2010) [12] reported that C. scolymus is rich in caffeoylquinic acid derivatives (cynarin and chlorogenic acid), flavonoids, volatile oils, phytosterols and tannins. The antioxidant activity of flavonoids have been reported to scavenge free radicals thus prevent the interaction of excess NAPQI with cellular DNA [13].Several research works reported that hepatoprotective effect of Artichoke is due to antioxidant activity of its chlorogenic acid and cynarin content [14]. Cynara scolymus leaves extract have long been used in folk medicine for their choleretic and hepatoprotective activities, that are often related to the cynarin content [15].Artichoke (Cynara scolymus L.) is an herbaceous perennial plant native to the Mediterranean area that belongs to the Asteraceae family[16] .The major chemical components of artichoke leaves include up to 2% phenolic acids with mono-and dicaffeoylquinic acids, primarily chlorogenic acid, cynarin, and caffeic acid. Also up to 0.1-1% flavonoids [17].

-Materials and Methods

- Chemicals: Paracetamol 500 mg tablet was obtained from the Essential Drug Company (Baghdad,Iraq), and given orally at a dose of 500 mg/kg body weight . Artichoke was purchased from local market (Kerbala, Iraq). Artichoke extract was extracted from its vegetative parts. Artichoke was given by gavages at a dose of 5oo mg /kg.

- Preparation of Plant's Extract: The dry plants materials were powdered and extracted with 70% aqueous ethanol using percolation method at room temperature. The extracts were filtered through Whatman no. 1 filter paper and evaporated to dryness under reduced pressure at a maximum of 40^0C using a rotary evaporator instrument [18].

- Animals: In this study, we used 20 female Wistar albino (230-250 g) rats obtained from animal house of Kerbala university/pharmacy college which were housed in groups in plastic cages, standard diet, tap water and placed on a 12-hour light/dark cycle for 28day (the experimental period).The animals were randomly divided into four groups, each experimental group consisted of 5 animals:

Control group, they were fed with only standard diet, glucose water and tap water for 28day. **Artichoke group**, rats were treated with artichoke with a dose of 500mg/kg daily orally via needle gavage started from the first day to last day of the experimental. **Paracetamol group, rats** were treated with 500 mg/kg paracetamol daily orally started from the 22 day to last day of the experimental. **Artichoke-Paracetamol group,** rats were treated with artichoke with a dose of

500mg/kg orally using needle gavage while paracetamol with dose of 500mg/kg was given orally via gavage daily started from the 22 day to last day of the experimental. After the last artichoke and paracetamol received,5 ml of blood samples were collected from each rat in centrifuge tubes. Serum was separated from coagulant blood by centrifugation and then frozen for biochemical analysis.

- Biochemical assay methods:

- Measurement of serum GPT ,Got ,Total protein ,bilirubin , glucose.

- Preparation of Liver Tissues for Histopathology

The specimens were fixed in formalin 10 % solution for 72 hours. After fixation, the tissues were washed under running tap water for 24 h and dehydrated with 50, 60, 70, 80, 90, 96 and 100% concentrated ethanol. The specimens were then laid in a 1:1 ratio of immersion oil and absolute alcohol for 1h, followed by immersion oil overnight, for transparency. After the application of xylol, the specimens were made into paraffin blocks using a 1:1 xylol and paraffin mixture for 1h and paraffin for 6 hours in an incubator. 10 micron thick sections were rehydrated and dyed with Masson's trichrome (Bio-Optica Masson tricromica cat no 04-010802, Milano S.p.a, via San Faustino, 58,20134 Milano, ITALIA) technique.

-Statistical Analysis:

Data were expressed as mean. Differences between control and other groups were tested for statistical significance using one-way analysis of variances (ANOVA). P-values of 0.05 or less were considered significant, statistical analysis was performed using SPSS for windows version (SPSS, Inc., Chicago, Illinois).

-Results:

Table 3-1 illustrates a significant increase in the level of serum bilirubin and glucose $p \leq 0.05$ in group treated with paracetamol and serum glucose $p \leq 0.05$ significantly increased in group treated with artichoke +paracetamol , while total serum protein $p \leq 0.05$ significantly decreased in group treated with paracetamol only as compared all groups with control group.

Groups	Bilirubin (mg/dl)	Glucose (mg/dl)	Total protein(mg/dl)
Control	0.313	119.62	7.1
Artichoke	0.312	123.07	7.07
Paracetamol	0.686^{a}	192.72^{a}	5.4^{b}
Artichoke+Paracetamol	0.389	143.35^{a}	6.8
LSD	0.1	5.99	0.5

- Different small letter means significant changing.
- a means significant increase.
- b means significant decrease.
- $p \leq 0.05$.

Table 3-2 illustrate a significant increase in the level of serum GOT and GPT $p \leq 0.05$ in group treated with **paracetamol** and **artichoke+paracetamol** only as compared all groups with control group.

Groups	GOT(U/L)	GPT(U/L)
Control	33.8	29.2
Artichoke	32.7	29.4
Paracetamol	164.5[a]	118.1[a]
Artichoke+Paracetamol	39.7[a]	41.85[a]
LSD	2.55	4.6

- Different small letter means significant changing.
- a means significant increase.
- b means significant decrease.
- $p \leq 0.05$

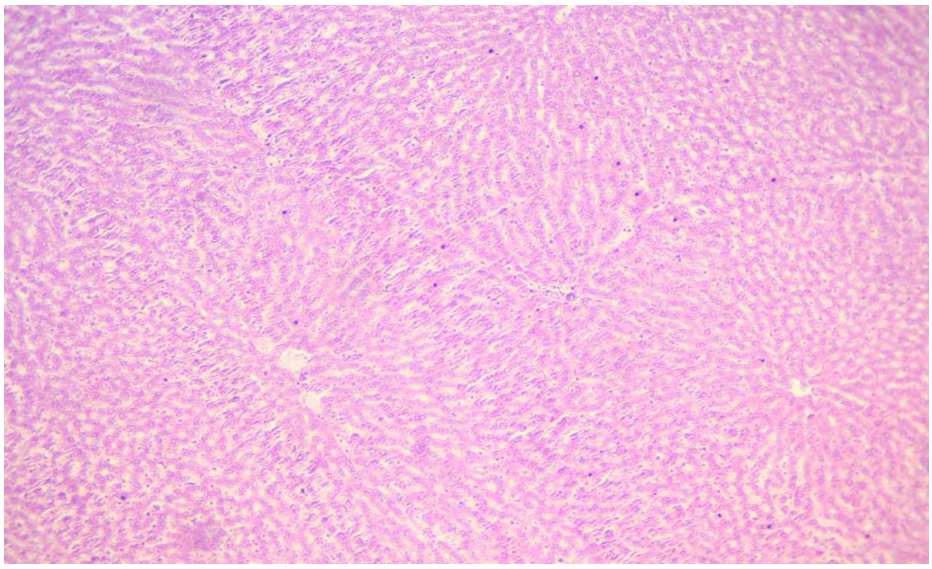

Figure 3-1 Liver section from a control rat showing Normal liver's parenchymal tissue.

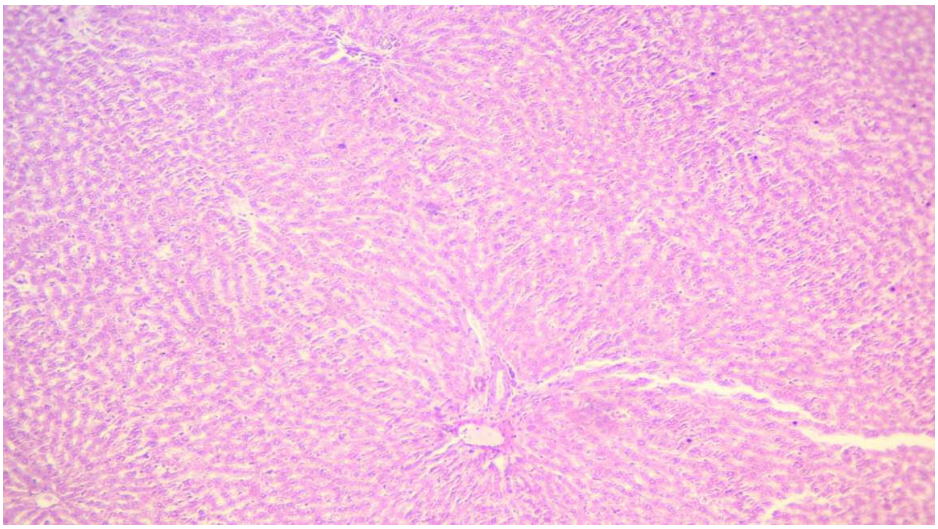

Figure 3-2 Liver section from rat treated with artichoke showing normal hepatocyte and no inflammatory cell.

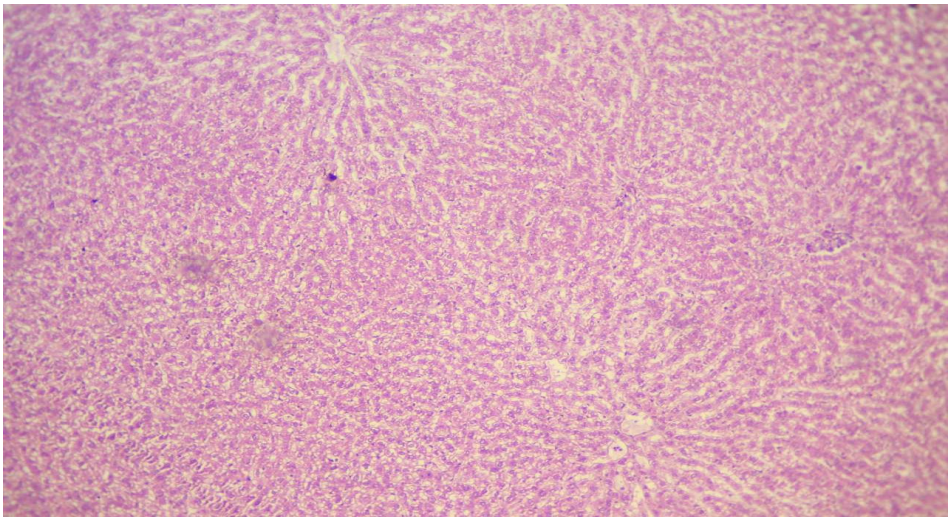

Figure 3-3 Liver section from rat treated with artichoke prior to and during treatment with paracetamol, the section shown slight congestion in central vein and reduction of inflammatory cells , degeneration in hepatocyte ,enlargement of sensoides , necrosis and odema .

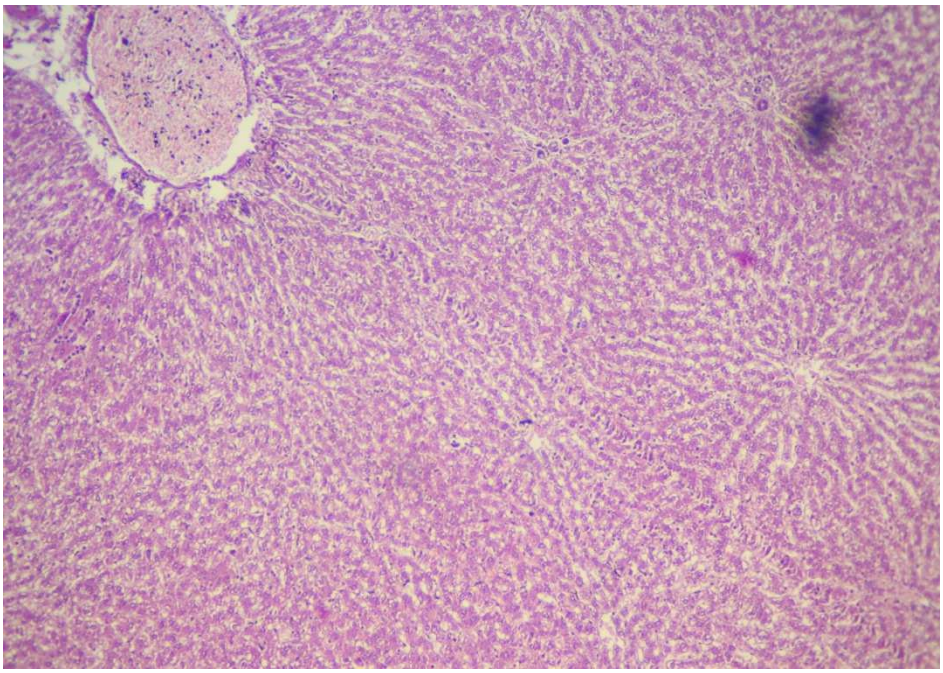

Figure 3-4 Liver sections from rat treated with paracetamol alone showing severe congestion in central , inflammatory cells , degeneration of hepatocyte , enlargement of sensoides , necrosis and odema .

-Discussion : Administration of paracetamol in higher doses (500 mg \kg) for 22 days caused a significant elevation of the serum level of liver function markers such as AST, ALT, bilirubin level in rats -paracetamol group as compared to control group (where it was found that control group s have normal liver's paranchymal tissue as seen in figure (3-1) has been attributed to the damage structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity as seen in figure four and five where there is severe congestion in central vein and there is inflammatory cell and degeneration in hepatocyte enlargement in sinusoids and necrosis and odema has been observed. [19].AST predominantly found in mitochondria of hepatocytes. The ALT, AST activity, serum bilirubin, and glucose levels are largely used as most common biochemical markers to evaluate liver injury, while total serum protein significantly decreased. [20] , high doses of Paracetamol lead to liver GSH depletion(due to conjugation of GSH with NAPQI to form mercapturic acid) subsequently leads to increased lipid peroxidation by abstracting hydrogen from a polyunsaturated fatty acid and ultimately, liver damage due to higher doses of paracetamol. [21] ,However, the administrations of artichoke extract along with Paracetamol have prevented the increased serum marker enzymes AST, ALT, glucose and bilirubin levels. This is in agreement with the commonly accepted view that serum levels of AST, ALT , glucose and bilirubin levels return to normal(protective effects of artichoke extract) with the healing of hepatic parenchyma and the regeneration of hepatocytes as seen in figure two where the severe congestion in central vein became slightly congestion when artichoke given alonge with paracetamol. [22]. cynarin has shown significant protecting and regenerating effects in the liver. It stimulates the clearance of bile from the liver, preventing congestion in the liver and diminishing the chances of liver damage as seen in figure three, where the hepatic function is normal and no inflammatory cells are present. Chlorogenic acid (*trans*-5-O-caffeoyl-D-quinic acid) is the quantitatively predominant hydroxycinnamate contained in artichoke tissues, and is directly involved in many of its well-documented hepatoprotective effects. [23]..Administration of artichoke extract significantly reduced the activity of liver enzymes in paracetamol induced rats due to its ability to reduce free radical-induced oxidative damage in the liver{ as observed in figure three} (reactive oxygen species such as superoxide and hydrogen peroxide) , artichoke extract has been shown to decrease liver enzymes in serum and prevent liver damage of rats with liver fibrosis, gallbladder bile stimulation, [24] ,There was a significant decrease in the concentration of serum total protein in paracetamol group as compared to control group, might be

depressed as a result of defective protein synthesis(The reactive oxygen species and their reaction products, such as the hydroxyl radical, are very harmful to cells,. [25] .The increasing liver damage also alters biochemical markers of liver function; International normalized ratio (INR) and the hepatic transaminases alanine transaminase, aspartate transaminases rise to abnormal levels. There was a significant increase in levels of serum ALT,AST, glucose, and bilirubin in paracetamol group as compared to control group .[26].Acetaminophen toxicity, tissue trauma, ischemia-reperfusion, sepsis, and other pathophysiological events activate both neutrophils and Kupffer cells directly or through activation of complement .Kupffer cells release cytotoxic mediators, such as reactive oxygen species, and proinflammatory mediators, such as cytokines and chemokines. Glutathione (GSH) is a critical cellular antioxidant. After GSH depletion the toxicity of acetaminophen was strikingly enhanced. [27].

Conclusion :From this study we can conclude there are a hepatoprotective and prophylactic effects of artichoke against paracetamol induce hepatic damage ,In the present study, we have observed that reduced GSH level was depleted significantly in the liver tissue of paracetamol treated rats compared to control group. Co-treatment with the artichoke extract had significantly improved level of GSH compared towards their control.

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