

The Possible ameliorative effect of *Cynara cardunculus* extract against liver injury and oxidative stress induced by acetaminophen in male albino rat *Rattus norvegicus*.

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Abstract: The present study was designed to evaluate the influence of *Cynara cardunculus* extract treatment against Paracetamol induced acute liver damage on albino rats. One hundred and twenty adult male rats were used in this study and distributed into fourteen groups (n=8). Animals of group 1 were treated with 1 ml of distilled water and served as control. 2nd group: Animals were received *Cynara cardunculus* extract (0.25gm/kg b. wt). 3rd group: Animals were received *Fraction 1 of Cynara*. 4th group: Animals were received orally *Fraction 2 of Cynara*. 5th group: Animals were received *Fraction 3 of Cynara*. 6th group: Animals were received *Fraction 4 of Cynara*. 7th group: Animals were received *Fraction 5 of Cynara*. 8th group: Animals were received a single oral dose of Paracetamol (Acetaminophen, N-acetyl-p-aminophenol) APAP (2g/kg. b. wt.) suspended in gum tragacanth (4%). Then animals were sacrificed 48 h after APAP administration. Groups from 9 to 14 were received a single oral dose of APAP (2gm/kg.b.wt) and after 48 h the animal co-administrated for 4 weeks daily as the following: 9th group: (APAP + *Cynara* treated group). 10th group (APAP + *Fraction 1 of Cynara* treated group). 11th group (APAP + *Fraction 2 of Cynara* treated group). 12th group (APAP + *Fraction 3 of Cynara* treated group). 13th group (APAP + *Fraction 4 of Cynara cardunculus* extract treated group). 14th group (APAP + *Fraction 5 of Cynara* treated group). The groups treated with Paracetamol showed elevation in ALP, total cholesterol, triglycerides, creatinine and depleted tissue GSH and increased the lipid peroxidation. Upon administration of paracetamol (2g/kg b.wt.) to albino rats. The result indicated that the extract of leaves of *cynara cardunculus* at 0.25g/ kg doses significantly reduced the elevated levels of biochemical markers mentioned above. Test extract treatment also increased the level of tissue GSH and significantly decreased tissue lipid peroxidation. In conclusion, this study suggests that *Cynara cardunculus* may have the potential therapeutic value in the treatment of paracetamol induced hepatic damage and some liver diseases. Hepatoprotective activity of the study plant may be attributed to the anti-oxidant principles in it.

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1. Introduction

Herbal medicines are being increasingly utilized to treat a wide variety of diseases, though the knowledge about their mode of action is relatively scanty. So there is a growing interest regarding the pharmacological evaluation of various plants used in traditional system of medicine. Many diseases (hepatitis, atherosclerosis, diabetes mellitus, asthma, nephritis) are due to the specific organ damage. The organ damage may be due to the excessive generation of free radicals (Kaushik et al., 2010).

Liver is the largest and almost complex organ in the body. It plays an important role in the maintenance of internal environment through its multiple and diverse function. It is involved in the intermediately metabolism of protein, fat and carbohydrates and also it synthesis number of plasma proteins. It is plays a central role in detoxification and excretion of many endogenous and exogenous compounds. Hence, any injury to it or impairment of its function has serious

implication for the health of the affected person (Sundari et al., 2013).

Paracetamol is widely used analgesic and antipyretic, produces acute liver damages at very larger dose. The hepatotoxicity of paracetamol has been attributed to the formation of toxic highly reactive metabolite n-acetyl parabenzoquinimine (NAPQI), which causes oxidative stress and glutathione depletion (Shah and Deval, 2011). It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Hurkadale et al., 2012).

Cynara is a relatively small genus, originating from the Mediterranean area that includes two crops, globe artichoke (*Cynara cardunculus* var. *Scolymus* L.) and cardoon (*Cynara cardunculus* var. *altilis* DC), as well as their ancestor, wild cardoon {(*Cynara cardunculus* L. var. *Sylvestris* (Lamk)) (Portis et al., 2005; Ierna and Mauromicale, 2010). Efterpi et al., 2012 reported that traditionally these crops have long been used as food and medicine. Nowadays,

they are considered as functional foods; owing to their nutritional properties. *Cynara cardunculus* has exhibited hepatoprotective and antioxidative activities, as well as the ability to inhibit cholesterol biosynthesis and low density lipoprotein oxidation.

2. Material and Methods

Animals

Using one hundred and twenty (112) clinically healthy mature adult male albino rats, the animals divided into (14) groups (n=8). The animals were obtained from the Animal House of the Faculty of Veterinary Medicine, Zagazig University, their weights ranged from (200-250gm), the animals were housed in standard conditions, where the animals were housed in metal cages and bedded with wood shavings and kept under standard laboratory conditions of aeration and room temperature at about 25°C. The animals were allowed to free access of standard diet and water *ad-libitum*.

Plant material

The plant material of *Cynara cardunculus* was collected in April 2011 from the private garden of (Faculty of Pharmacy, Zagazig University) and it was identified by Prof. Dr. Samih Ibrahim El-Dahmy Professor of Pharmacognosy, Faculty of Pharmacy, Zagazig University.

Extraction:

Fresh cut aerial parts (leaves and stems) were extracted by maceration in hot ethyl alcohol 96%. For the botanical study, fresh samples were used.

Column chromatography: Total extract was fractionated to 5 Fractions

Solvent system: Fraction1 (100% L.P.), Fraction2 (L.P.: CHCl₃ 8:2), Fraction3 (L.P.:CHCl₃ 7:1), Fraction4 (CHCl₃ (100%) and Fraction5 (CHCl₃: MeOH 1:1).

Drugs:

Paracetamol (Acetaminophen, N-acetyl-p-aminophenol) (APAP) is a derivative of para-aminophenol, was purchased from the local pharmacy. It is manufactured by Glaxo Wellcome Co. Cairo, Egypt, Each tablet contains 500 mg, tablets were then suspended in distilled water and gum tragacanth (5%) to form a suspension, then the drug was given orally in a dose level of (2g/Kg b. wt.), the dose administration is as follow: (2g of suspension is equivalent to 1 ml of prepared solution) which is equivalent to the daily therapeutic dose in human.

Animal Treatment

Animals were divided into twenty-two groups (each group contain 8 rats). Groups from 1 to 8 were treated orally using metallic stomach tube daily for 4 weeks as following:

1st group: (Control group):

Animals received 1ml of gum tragacanth (4%).

2nd group:Animals were received *Cynara cardunculus* extract (0.25gm/kg b. wt).

3rd group: Animals were received *Fraction 1 of Cynara* (0.25g/kg b. wt).

4th group:

Animals were received orally *Fraction 2 of Cynara* (0.25g/kg b. wt).

5th group:

Animals were received *Fraction 3 of Cynara* (0.25g/kg b. wt).

6th group:

Animals were received *Fraction 4 of Cynara* (0.25g/kg b. wt).

7th group:

Animals were received *Fraction 5 of Cynara* (0.25g/kg b. wt).

8th group: Animals were received a single oral dose of APAP (2g/kg. b. wt.) suspended in gum tragacanth (4%).Then animals were sacrificed 48 h after APAP administration.

Groups from 9 to 14 were received a single oral dose of APAP and after 48 h the animal co_adminstrated for 4 weeks daily as the following:

9th group: (APAP + *Cynara* treated group).

10th group :(APAP +*Fraction 1 of Cynara* treated group).

11th group: (APAP +*Fraction 2 of Cynara* treated group).

12th group :(APAP +*Fraction 3 of Cynara* treated group).

13th group: (APAP +*Fraction 4 of Cynara* treated group).

14th group: (APAP +*Fraction 5 of Cynara* treated group).

Blood sampling:

Blood samples were collected from the retro-orbital vein, which is a simple, convenient and successful procedure that allows bleeding of the same animal more than one time with minimal stress (*Scherener, 1967*).

Statistical analysis:

Data were collected, arranged, summarized and then analyzed using the computer program SPSS/PC+ (2001) The statistical method was one way ANOVA test (t-test), LSD (Least significant difference) according to (*Snedecor and Cochran, 1982*) to estimate the effect of different treated groups.

3. Results

Biochemical markers

Fraction 1 proved that has potent effect. Paracetamol elevated the levels of serum enzymes (SGPT, SGOT, ALP, and creatinine) caused very high significant ($P<0.001$) increase. This indicates that paracetamol treatment caused liver damage. However Table (1) showed very high significant ($P<0.001$) decrease in the level of biochemical parameters as a result of the effect of paracetamol combination with all other treatment when

compared with paracetamol group. Paracetamol elevated the levels of serum total cholesterol, serum triglycerides caused very high significant ($P < 0.001$) increase. This indicates that paracetamol treatment caused liver damage. Paracetamol combination with Cynara did not cause any significant ($P > 0.05$) change in the levels while the Paracetamol combination with Fraction-1 elicited

significant ($P < 0.05$) increase in the levels in table (2).

In spite of, this decrease not returned to the normal values of enzyme levels, but the highest decrease was cleared as a result the effect of paracetamol combination with cynara and with fraction-1 (.25gm/ kg).

Table (1): Effect of paracetamol and paracetamol combination with each of Cynara and Cynara fraction-1 on biochemical parameters in male albino rats.

Groups	Statistical Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)	Creat. (mg/dl)
Control	Mean \pm S.E	43.17 \pm 1.400	48.83 \pm 1.447	74.33 \pm 1.081	0.450 \pm .0428
Paracetamol	Mean \pm S.E % of change P-value	165.33 \pm 5.327 +283.0% < 0.001***	164.17 \pm 3.646 +236.2% < 0.001***	132.3 \pm 2.459 +77.99% < 0.001***	0.917 \pm .0477 +103.7% < 0.001***
Paracetamol + Cynara	Mean \pm S.E % of change P-value	42.17 \pm 1.956 -2.32% > 0.05 ^{ns}	49.00 \pm 0.966 +0.35% > 0.05 ^{ns}	77.67 \pm 0.955 +4.49% > 0.05 ^{ns}	0.480 \pm .0365 +4.1% > 0.05 ^{ns}
Paracetamol + Fraction-1	Mean \pm S.E % of change P-value	43.83 \pm 2.136 +1.53% > 0.05 ^{ns}	50.83 \pm 1.276 +4.10% > 0.05 ^{ns}	77.17 \pm 1.600 +3.82% > 0.05 ^{ns}	0.488 \pm .0393 +5.81% > 0.05 ^{ns}

^{ns} :non significant

*** :very highly significant

GSH level

There was a marked depletion of GSH level in paracetamol treated group very high significant ($P < 0.001$) decrease. *Cynara cardunculus* and Fraction-1 of *Cynara cardunculus* extract 0.25gm/kg showed a dose dependent and very highly significant ($P < 0.001$) increase in levels of tissue GSH. The results are compiled in Table. (2).

Lipid peroxidation

The effect of *Cynara* on lipid peroxidation in paracetamol induced liver damage shown showed very highly significant ($P < 0.001$) increase in table (2). Treatment with *Cynara cardunculus* and Fraction-1 of *Cynara cardunculus* extract 0.25gm/kg extract showed very high significant ($P < 0.001$) decrease the lipid peroxidation in a dose dependent manner.

Table (2): Effect of paracetamol and paracetamol combination with each of Cynara and Cynara fraction-1 on GSH and Lipid peroxidation in male albino rats.

Groups	Statistical Parameters	MDA (mmol/mg protein)	GSH (mg/mg protein)	Trig. (mg/dl)	T. Chol. (mg/dl)
Control	Mean \pm S.E	.2583 \pm .0060	7.417 \pm .0872	98.50 \pm 0.992	77.67 \pm 0.989
Paracetamol	Mean \pm S.E % of change P-value	.4950 \pm .0248 +91.63% < 0.001***	4.433 \pm .0882 -40.23% < 0.001***	154.83 \pm 1.515 +57.18% < .001***	166.67 \pm 1.926 +114.5% <.001***
Paracetamol + Cynara	Mean \pm S.E % of change P-value	.2600 \pm .012 +0.658% > 0.05 ^{ns}	6.980 \pm .1091 -5.617% > 0.05 ^{ns}	103.00 \pm 1.461 +4.6% > 0.05 ^{ns}	80.37 \pm 0.94 +5.79% > 0.05 ^{ns}
Paracetamol + Fraction-1	Mean \pm S.E % of change P-value	.2717 \pm .013 +1.67% > 0.05 ^{ns}	7.090 \pm .1106 -5.826% >0.05 ^{ns}	104.47 \pm 1.579 +6.17% < 0.05*	83.67 \pm 1.745 +9.2% < 0.05*

^{ns} :non significant

* :significant

*** :very highly significant

4. Discussion

Biochemical markers

In the present study the damage of liver due to Paracetamol over dosage (2 g/kg b.wt.) was confirmed by elevated levels of biochemical

parameters like ALT, AST and ALP to 165.33, 164.17 and 132.3 respectively when compared with control group. This may be due to the fact that hepatic cells possess a variety of metabolic activities and contain a host of enzymes. ALT and AST found in higher concentration in cytoplasm and mitochondria. In liver injury the transport function of hepatocytes is disturbed, resulting in the leakage of plasma membrane (*Rajesh and Latha 2004 and Kaushik et al., 2010*)

The obtained results revealed that the oral treatment of Paracetamol-treated rats with separately daily dose of *Cynara cardunculus* extract and *Cynara* Fractions (1 – 5) for 28 days caused a significant decrease in the elevated levels of AST, ALT and ALP the best results was obvious with *Cynara*, *Cynara* Fraction-1.

This may be due to that the *Cynara* extracts and its Fraction-1 contain compounds that can be useful in the treatment of liver damage as phenolic compounds and flavonoids. Some studies describe numerous pharmacological activities associated to cardoon such as hepatoprotective, antioxidative, anticarcinogenic, hypocholesterolemic, antibacterial, anti-HI, bile-expelling and urinate effects (*Kukic et al., 2008; Lattanzio et al., 2009; Shen et al., 2010 and Sandra and Paula, 2012*)

In this study, the treatment of rats by single oral dose of Paracetamol (2 g / kg.b.wt) caused very high significant ($P < 0.001$) increase in the serum levels of triglycerides, total cholesterol and creatinine to 154.83, 166.67 and 0.917 respectively.

The Paracetamol toxicity responsible for production of oxidative stress, which is the key contributor in hepatic injury and it is known to produce reactive oxygen species (ROS) that is responsible for significant change in Lipid profile and hepatic dysfunction and more critical consequences. These suggestions were in full agreement with *Kaushik et al. (2010)* they reported that the liver is a major organ system involved in the metabolism of various drug, xenobiotics and toxins. During the metabolism, excessive free radicals are generated and may cause liver damage. The Paracetamol produces acute liver damage at very larger dose. The hepatotoxicity of Paracetamol has been attributed to the formation of toxic highly reactive metabolite NAPQI. They also established that the total cholesterol and serum triglycerides level also increased in Paracetamol induced liver damage. The total cholesterol level increased may be due to the inhibition of destruction of triglycerides secretory mechanism by liver.

GSH level and Lipid peroxidation

In the current study, the daily treatment of rats by daily oral dose of each *Cynara* and *Cynara* Fractions (1 to 5) for 28 days did not cause any significant change ($P > 0.05$) in all determined antioxidant parameters (GSH and MDA) compared

with control group, while treatment of rats by single oral dose (2 g/kg.b.wt) of Paracetamol caused very high significant ($P < 0.001$) decrease in CAT and GSH levels to 4.433, but caused very highly significant ($P < 0.001$) increase in MDA levels by percent change reach to .4950 compared with control group.

Paracetamol induced toxicity in rats is one of the widely used experimental model to evaluate the hepatoprotective nature of herbal extracts (*Franchesca et al., 2010 Madhu et al., 2012 and Nirmala et al., 2012*).

Paracetamol overdose results in metabolic activation of the drug to the reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI), which depletes glutathione GSH and forms acetaminophen protein adducts, triggering the initiation of the injury process with mitochondrial oxidative stress (*Moffit et al., 2007 and Junfeng et al., 2013*).

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