Protective Effect of Cat's Claw against Acetaminophen-Induced Hepatotoxicity in Mice

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Abstract: Overdose of acetaminophen (ACT), a widely used analgesic drug, can result in severe hepatotoxicity and is often fatal. This study was undertaken to examine the effects of cat's claw (*Uncariatomentosa*, UT), which is a herbal medicine used widely to treat inflammatory disorders, on acetaminophen –induced hepatotoxicity in mice. Mice were given cat's claw (10 mg $kg^{-1}ip^{1}$) eight days before a hepatotoxic dose of acetaminophen (500 mg kg^{-1}) intraperitoneally. Acetaminophen hepatotoxic effect manifested by a significant increase in serum enzyme levels including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and total nitrate/nitrite. In addition, a significant increase in hepatic lipid peroxides and a significant decrease in hepatic reduced glutathione (GSH) content. More over the enzyme activities of glutathione peroxidase and superoxide dismutase in liver were significantly decreased. Histopathological finding also showed marked hepatic necrosis with acetaminophen. Interestingly, supplementation of UT for 8 days before acetaminophen administration completely reversed the biochemical and histopathological changes induced by ACT to the control values. In conclusion, UT is effective in protecting mice against acetaminophen-induced hepato toxicity possibly via increased resistance to oxidative and nitrosative stress.

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Key Words: Acetaminophen , cat's claw, hepatotoxicity , oxidative and nitrosative stress.

1.Introduction:

Acetaminophen (ACT), which is also named paracetamol, is a widely used antipyretic and analgesic drug and its overdose cause hepatotoxicity [1,2]. Although a large dose of acetaminophen is directly conjugated with glucuronic acid or sulfate and excreted, a significant amount of acetaminophen is metabolized by the cytochrome P450 system [3]. This leads to the formation of a reactive metabolite, presumably N-acetyl-p-benzoquinoneimine (NAPQI), which reacts rapidly with glutathione [3]. Thus, acetaminophen causes dramatic depletion of cellular glutathione levels in the liver [4]. If the formation of NAPQI exceeds the capacity of hepatocellular GSH, it will covalently bind to cellular proteins leading to hepatocellular injury [5].

The mechanism of hepatocellular injury after the initial NAPQI formation, glutathione depletion and covalent binding to proteins is still unclear. However, many studies have suggested that macrophages and formation of reactive oxygen and nitrogen species are involved in the development of toxicity of acetaminophen. Pretreatment of mice with gadolinium chloride or dextran sulfate to inactivate macrophages have been shown to dramatically decrease acetaminophen toxicity in mice [6–8]. In the acetaminophen-treated mice there was a direct correlation between nitric oxide synthesis, as measured by serum levels of nitrate plus nitrite, and hepatotoxicity [9]. It was also reported that toxic doses of acetaminophen lead to induction of the inducible nitric oxide synthase and that pretreatment of mice with a nitric oxide synthase inhibitor, aminoguanidine, decreases the toxicity [10].

Cat's claw (Uncariatomentosa, UT) is known with the common names cat's claw. The following properties are attributed to cat's claw: antibacterial antimutagenic, antioxidant, anti-inflammatory, antitumorous, antiviral, cytostatic, diuretic, hypotensive, immune-stimulant and vermifuge [11]. UT is used in traditional Peruvian medicine for the treatment of a wide range of health problems, particularly digestive complaints and arthritis. Among the numerous factors associated with liver inflammation the enhanced production of oxidants and free radicals have become widely recognized as integral components of cell and tissue injury [12-15]. For these reasons, it is important to search for complementary treatments, including phytotherapic plants, that minimize the liver damage associated with acetaminophen toxicity.

Since oxidative and nitrosative stress play an important role in acetaminophen-induced hepatotoxicity [16,17] and UT possess strong anti-oxidative as well as anti-nitrosative properties, therefore, it is reasonable to hypothesize that the use of UT could protect against acetaminophen-induced hepatotoxicity.

Aim of the work:

The aim of the work is to study the possible cyto-protective effect of cat's claw against acetaminophen induced hepatotoxicity.

2.Materialsand Methods

Chemicals and Drugs

Acetaminophen was purchased from Merck (Germany). Cat's claw was purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were of the highest grade commercially available.

Animals

Male Swiss albino mice weighing 22-25 g were used in all experiments. Animals were maintained under standard conditions of temperature & humidity with regular light/dark cycle and allowed free access to food (Purina Chow) and water. All animal experiments were conducted according to the regulations of the Committee on Bioethics for Animal Experiments of Riyadh colleges of dentistry and pharmacy.

Induction of liver damage

Acetaminophen group, was treated with a single dose of acetaminophen (500 mg kg-1 i.p) and killed after 24 hours. The rise in serum ALT was taken as evidence for impaired liver function. [18]

Experimental protocol:

Animals were randomly assigned to 4 groups of 10 each:

Group 1 (Control): daily intraperitoneal injections of isotonic saline (10 ml/kg) for 8 days.

Group 2 (UT): daily intraperitoneal injections of cat's claw (10 mg kg-1 ip) for 8 days (19)

Group 3 (ACT): a single dose of acetaminophen (500 mg kg-1 i.p.) and killed after 24 hours.

Group 4 (ACT + UT): daily intraperitoneal injections of cat's claw (10 mg kg-1 ip) for 8 days then given a single dose of acetaminophen (500 mg kg-1 i.p.).

At 24 h after last ACT injection, blood samples were drawn from the orbital plexus, under light ether anesthesia, into non-heparinized capillary tubes. Serum was separated by centrifugation for 5 min at 4000 rpm and stored at –20˚C until analysis. The liver was isolated, washed with saline, weighed, and then 10% (w/v) homogenate of the liver was made in ice cold saline.

Determination of serum enzymes ALT, AST and LDH

Serum ALT, AST and LDH were determined colorimetrically and kinetically as described by Bergmeyer et al., 1978 and Buhl& Jackson, 1978 respectively, using commercially available diagnostic kits (bioMērieux-RCS Lyon-France).[20,21]

Determination of total nitrate/nitrite (NO(x)) concentrations in serum

Total nitrate/nitrite (NO(x)) was measured as stable end product, nitrite, according to the method of Miranda et al. [22]. The assay is based on the reduction of nitrate by vanadium trichloride combined with detection by the acidic griess reaction. The diazotization of sulfanilic acid with nitrite at acidic pH and subsequent coupling with N-(10 naphthyl)-ethylenediamine produced an intensely colored product that is measured spectro-photometrically at 540 nm. The levels of NOx were expressed as mol g−1 wet tissue.

Determination of lipid peroxides, glutathione content and enzyme activities of Glutathione peroxidase andsuperoxide dismutase in liver homogenate:

Glutathione contents and lipid peroxidation (Malondialdhyde (MDA) production) in the hepatic tissues were determined according Ellman, 1959 and Ohkawa et al., 1979 respectively. [23.24] The *enzyme activity of* Glutathione peroxidase (GSH-Px) and superoxide dismutase were measured in the liver homogenates according Lawrence & Burk ,1978 and McCord & Fridovich,1969 respectively.[25,26]

Histopathology

Histological examination was performed on about 50% of randomized animals of each group. Liver samples were taken from the distal portion of the left lateral lobe. The tissue was fixed for at least 48 hours in 10% formalin. The samples were then embedded in paraffin, cut into 5 urn sections, and stained with hematoxylin and eosin for examination by Light micrograph.

Statistical analysis:

Data are expressed as mean + S.E. for the groups. Comparisons of parameters between different groups were evaluated by One-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Results considered statistically significant when$P< 0.05$.

3.Results:

Effects of UT on ACT-induced changes in serum biochemical parameters:

Figure 1 shows the effects of ACT, UT and their combination on the indices of serum liver function, ALT, AST and LDH (Figs. 1,2,3). ACT resulted in a significant 450%, 303% and 149% increase in serum ALT, AST and LDH, respectively, as compared to the control group. Combined treatment ACT with UT leads to decrease significantly ($P<0.001$) the enzyme activities of ALT, AST and LDH as compared with ACT group.

Oxidative and NitrosativeStress Biomarkers

Figs. 4,5,6 show the effects of ACT, UT and their combination on oxidative and nitrosative stress biomarkers namely thiobarbituric acid reactive

substance (MDA), GSH in hepatic tissues and total nitrate/nitrite in serum (NOx) respectively. ACT resulted in a significant 57% decrease in GSH, a significant 225% increase in MDA and a significant 141% increase in NOx as compared to the control group. Combined ACT treatment with UT decreased significantly MDA and NOx (P<0.01) and restore GSH level in hepatic tissues compared to the control values.

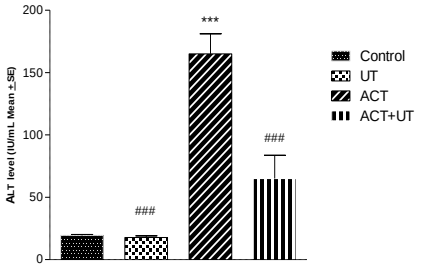
Antioxidant Enzyme Activities

Figs. 7and 8 show the effects of AST,UT and their combination on the activity of antioxidant enzymes Gpx and SOD in hepatic tissues respectively. ACT resulted in a significant decrease in both Gpx and SOD enzyme activities as compared to the control gro (*both P<0.01*). Combined ACT treatment with UT improve significantly both enzymes activity (*P<0.05*) inhepatic tissues compared with ACT group.

Histopathological study:

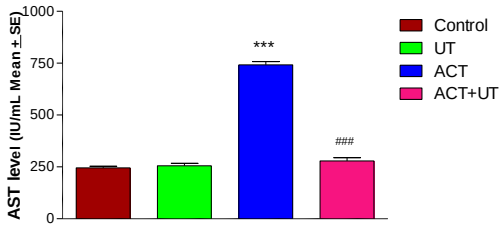
Combined treatment ACT with UT completely protected the liver against ACT-induced hepatic necrosis (Fig. H-2) and no signs of hepatic damage were observed. Mice treated with ACT showed extensive hepatocellular damage, vacular degeneration together with central zonal necrosis after 24 hours from the last injection(Fig. H-1). Control mice had normal hepatic architecture.

Fig (1) Effects of UT on elevated serum enzymes ALT activities induced by ACT



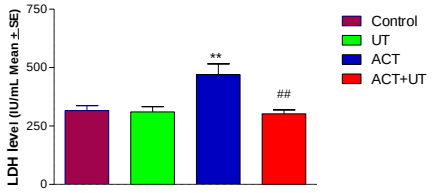
UT (10 mg/kg/day i p.) was given for 8 day before and during the experimental period ,while ACT (500mg/kg i.p.) injected.
· Significantly different from control group # Significantly different from ACT
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (2) Effects of UT on elevated serum enzymes AST activities induced by ACT



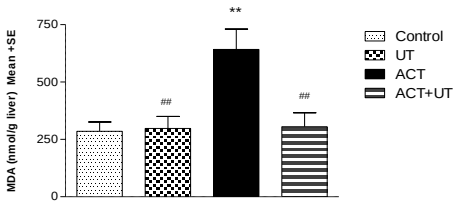
UT (10 mg/kg/day i.p.) was given for 8 day before and during the experimental period ,while ACT (500mg/kg i.p.) injected.
· Significantly different from control group # Significantly different from ACT
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (3) Effects of UT on elevated serum enzymes LDH activities induced by ACT



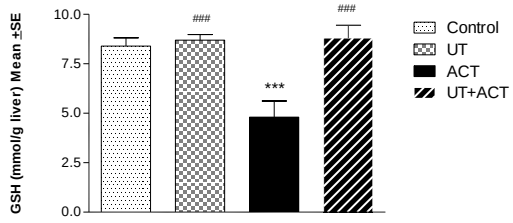
UT (10 mg/kg/day i.p) was given for 8 day before and during the experimental period ,while ACT (500mg/kg i.p.) injected.
· Significantly different from control group # Significantly different from ACT
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (4) Effect of ACT, UT and their combination on the levels of thiobarbituric acid reactive substance (MDA) in mice hepatic tissues



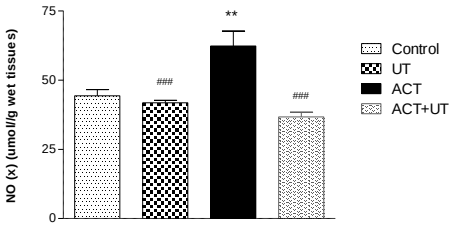
UT (10 mg/kg/day i.p.) was given for 8 day before and during the experimental period ,while ACT (500mg/kg i.p.) injected.
· Significantly different from control group # Significantly different from ACT
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (5) Effect of ACT, UT and their combination on the levels of reduced glutathione in mice hepatic tissues



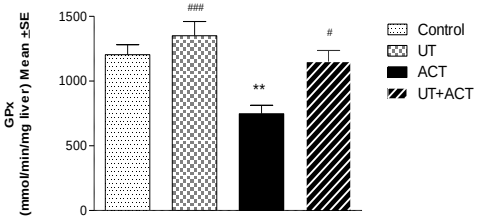
UT (10 mg/kg/day i.p.) was given for 8 day before and during the experimental period ,while ACT (500mg/kg i.p.) injected.
· Significantly different from control group # Significantly different from ACT
#* P<0.05 ##** P<0.01 ###**** P<0.001

Fig (6) Effect of ACT, UT and their combination on the levels of total nitrate/nitrite in serum



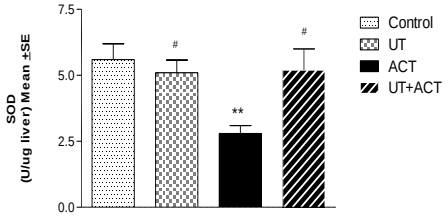
UT (10 mg/kg/day i.p.) was given for 8 day before and during the experimental period ,while ACT (500mg/kg i.p.) injected.
· Significantly different from control group # Significantly different from ACT
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (7) Effect of ACT, UT and their combination on liver glutathione peroxidase activity



UT (10 mg/kg/day i.p.) was given for 8 day before and during the experimental period ,while ACT (500mg/kg i.p.) injected.
· Significantly different from control group # Significantly different from ACT
#* P<0.05 ##** P<0.01 ###*** P<0.001

Fig (8) Effect of ACT, UT and their combination on liver superoxide dismutase activity



UT (10 mg/kg/day i.p.) was given for 8 day before and during the experimental period ,while ACT (500mg/kg i.p.) injected.
· Significantly different from control group # Significantly different from ACT
#* P<0.05 ##** P<0.01 ###*** P<0.001

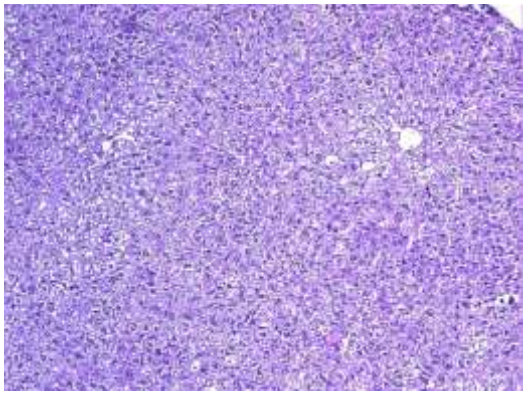


Fig. H 1: A photomicrograph of liver of control group showing normal hepatic structre. (H&E...x200)

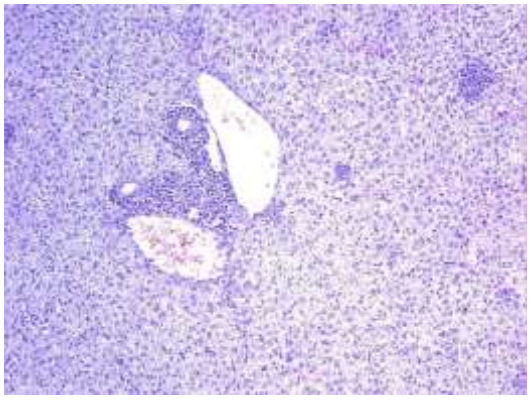


Fig. H2: A photomicrograph of liver of ACT-treated group showing severe inflammatory infiltrates mainly lymphocytic cells (H&E...x200).

4. Discussion

The obtained results showed that the serum aminotransferase activity was significantly elevated after ACT administration. This indicates that ACT induced liver injury in the early phase of acute intoxication. In addition, the liver MDA concentrations were significantly higher in ACT group in comparison with those of the control group. Based on these data, it could be suggested that lipid peroxidation may be an important mechanism of ACT-induced hepatotoxicity, even when administered acutely. Lipid peroxidation was confirmed to be an important mechanism of ACT-induced liver injury at different doses in various studies. [27-31] The role of lipid peroxidation was also confirmed by the protective effects of L-carnitine and some medicinal plants on hepatocyte injury caused by ACT.[28,29.30] This protection can, at least partly, be explained by their ability to inhibit MDA production. Similarly, earlier studies showed that a vitamin E-rich diet reduces lipid peroxidation in hepatocytes due to ACT toxicity. Vitamin E was found to protect animals against ACT hepatotoxicity. [28,32,33]

Serum nitrites and nitrates, markers of NO production, were increased in ACT group. These findings suggest that reactive nitrogen species are also involved in acute ACT- induced liver injury. The role of nitrosative stress in acute ACT intoxication, detected as an increase in nitrotyrosine production, was suggested in various studies. [34-36] Nitrotyrosine immune-reactivity was found to be increased 4 h after ACT treatment in mouse liver. [34]

Similarly, it was reported that in low concentrations NO exerts hepatoprotective effects, while in high concentrations, NO in the presence of superoxide anion leads to peroxynitrite formation, which is known to be a potent oxidant in cells. [28] Normally, peroxynitrite is detoxified by GSH/GSH peroxidase, especially in the mitochondria. [37] It has been postulated that GSH depletion induced by ACT leads to peroxynitrite accumulation, thus aggravating nitrosative stress.48 The present results are in accordance with these data, since a significant GSH depletion was found in ACT group.

Various studies suggested that GSH depletion plays a contributory role in ACT hepato-toxicity.[39,40] *N*-Acetylcysteine, a GSH precursor, was found to reduce ACT toxicity in mice and this effect was pronounced in the first hours after ACT treatment.[41] Similarly, pretreatment with α-lipoic acid reduced GSH depletion induced by high doses of

ACT and protected markedly against its hepatotoxicity. [42] In addition, *in vitro* studies confirmed a profound GSH depletion after a ACT overdose. [43] This is not surprising since the major mechanism involved in early ACT hepatotoxicity is inactivation of sulfhydryl groups of various cellular compounds due to NAPQI detoxification.[28]

Liver SOD activity was decreased after administration of ACT. This decrease liver SOD activity was reported in various experimental models.[44,45]The simultaneous induction of glutathione peroxidase after UT treatment is largely contribute to the increased resistance of the liver cells to ACT toxicity as well as intracellular ROS accumulation . Furthermore, the induction of GPx by UT may lead to increased regeneration of GSH from GSSG produced during GPx-catalyzed decomposition of H202 in liver cells. Several recent studies have also reported that GPx plays an important role in protecting cells against ROS- mediated injury. [28]

Uncariatomentosa(*UT*, Cat's claw) has antioxidant properties [11] and can stimulate DNA repair [46] and myelopoiesis [47]. Eberlin*et al.*[47] showed that UT extract promotes proliferation of myeloid precursors through the increase in serum colony stimulating growth factors (CSFs). Other experiments have demonstrated the positive effect of UT on leukocyte counts over a period of eight weeks in healthy animals [11] and after ten days of doxorubicin-induced neutropenia. [48]

Many previous studies have clearly shown the potential of the antioxidant UT, and its potent radical scavenger activity was confirmed by several assays including the following: the capacity to reduce the free radical diphenylpycrilhydrazyl (DPPH assay) [49, 50], the reaction with the superoxide anion, peroxyl [49], and hydroxyl radicals [49] as well as with the oxidant species, hydrogen peroxide, and hypochlorous acid assay. [49, 51]

In summary, administration of UT protected mice from acetaminophen-induced hepatotoxicity. The protection is not through the change in metabolism of acetaminophen but may be due to reduction of oxidative and nitrosative stress. These protective effects of UT on liver injury might have a considerable impact on developing clinically feasible strategies to treat patients with drug induced hepatitis.

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