Histopathological study on the protective effect of vitamin C against paracetamol-induced acute hepatic damage in rat.

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Abstract:
Paracetamol (PCM) as analgesic drugs makes hepatotoxicity damage. Vitamin C (VC) has been recognized as an antioxidant and has shown protective effects against hepatotoxicity damage caused by paracetamol. The current investigation aims to study the possible protective effects of VC against hepatic damage induced by PCM in rats. Forty male rats were divided into five groups: The primary group as control; receiving distilled water orally, the second group; receiving 500 mg/kg of VC. The third group; receiving 500 mg/kg of PCM. The fourth group (protective group); receiving VC for 7 days, and then PCM for another 7 days, and the last group treated with a combination of VC and PCM for 14 days. The liver tissues after administration of PCM alone showed different changes such as disrupted lobular architecture with degenerating hepatocytes, dilated congested central vein, and mononuclear cellular infiltration. While the protective group preserved the general architecture and lacked evidence of major morphological. Additionally, VC with PCM showed some alterations in the liver architecture. In conclusion, the results of this study
demonstrate that VC was effective in reducing the hepatic damage caused by PCM in male albino rats.

**Key Words:** Histopathology, Paracetamol, Vitamin C, liver, Rats.
Introduction:

Paracetamol (PCM) is one of the most common causes of poisoning worldwide [1]. Where most people are only at risk for liver toxicity; if they take more than the normal recommended amount of PCM [2]. A number of studies have demonstrated that high dose of PCM caused increase the levels of reactive oxygen species (ROS), therefore increasing oxidative stress and causing liver and renal damage [3]. PCM, also known as (acetaminophen or N-acetyl-p-amino-phenol) is the most commonly used antipyretic and pain reliever and since 1955 is a widely analgesic medication in many countries [4], [5]. An overdose of paracetamol is a frequent reason for liver and renal toxicity and possible death [6]. PCM is metabolized in the liver by cytochrome P450 (CYP450) enzymes, to N-acetyl-p-benzoquinone imine (NAPQI). NAPQI reacts with glutathione (GSH), therefore overdoses of paracetamol may result in a depletion of hepatocellular GSH [7], [8]. On the other hand, it has been revealed that CYP2E1, CYP1A2, and intracellular GSH take part an important role in the hepatotoxicity induced by paracetamol [9].

Antioxidants protect key cell components from damage by neutralizing the free radicals. Antioxidants that occur naturally in the body or that are consumed through the diet may block damage to cells [10]. Vitamin C (VC) (ascorbic acid) is a water-soluble micronutrient required for multiple biological functions [11], [12]. VC may prevent certain types of hepatic cellular damage [13]. VC is a natural antioxidant found in citrus, soft fruits, and leafy green vegetables [14]. It prevents the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues [13]. Therefore, there is a growing need for exogenous sources of antioxidants such as vitamins which have various biological activities. So, the present study examined the possible protective effects of VC on PCM-induced hepatic oxidative insult in rats.

Material and Methods:

Experimental chemicals:

1- VC (ascorbic acid C₆H₈O₆) (500 mg) was obtained from the pharmacy.

2- PCM (C₈H₉NO₂) (acetaminophen or N-acetyl-p-amino-phenol) (500 mg) was obtained from the pharmacy.
Experimental animals:

Healthy male albino rats (Rattus norvegicus) with an average weight of 200-250 g were used in this study were obtained from the Central Animal House, College of Veterinary, University of Omar Al-Mokhtar, El-Beida, Libya. All animals were allowed two weeks per-experimentation period to acclimatize to laboratory conditions in order to avoid any complications along the course of the experiment. They were housed in cages at room temperature. Rats were fed with laboratory diet and water ad libitum with fresh daily supplies.

Experimental design:

Forty male rats were randomized into five groups 8 rats in each:

Group (1): Normal control group (NC), animals were given distilled water orally by gavage for 14 days.

Group (2): VC treated group (VC), animals were given VC (500 mg/kg/b.w) according to Adeneye and Olagunju, [15] orally by gavage for 14 days.

Group (3): PCM treated group (PCM), animals were given PCM (500 mg/kg/b.w.) according to Modo et al., [16] orally by gavage for 14 days.

Group (4): Protective group (PRO), animals were given VC (500 mg/kg/b.w.) for 7 days then given PCM (500 mg/kg/b.w.) orally by gavage for 7 days.

Group (5): VC and PCM treated group (VC+PCM), animals were given VC (500 mg/kg/b.w.) before administration of PCM (500 mg/kg/b.w.) orally by gavage for 14 days.

All rats were received treatments six days a week [17]. At the end of the experimentation and 24 hours after the last dose, all animals were sacrificed, then the liver were removed.

Histopathological study:

Liver tissues specimens from all groups were fixed in formalin and embedded in paraffin. Sections of 5μm thickness were stained with hematoxylin and eosin using standard procedures [18].
The stained sections were examined under light microscope.

Changes in the experimental histopathologic parameters for liver tissues were graded as follows: (-) showing no changes, (+) (++), and (++) indicating minimum, moderate, and maximum changes, respectively [19].

Results:

Histopathological preparations of liver tissues:

Microscopically, the liver sections of the control group showed a normal lobular architecture (Figure. 1). The hepatocytes radiated from the central vein forming anastomosing plates of liver cells, separated from each other by vascular spaces, hepatic sinusoids. The hepatocytes appeared polyhedral with acidophilic cytoplasm and large central rounded vesicular nuclei with prominent nucleoli.

Light microscopic examination of the liver after administration of VC alone revealed a normal histological structure: Normal central vein, sinusoidal capillary size with no evidence of congestion or narrowing, and normal hepatocyte without any changes in their cytoplasm, and nucleus (Figure. 2) as in control group.

Histopathological examination of the liver after administration of PCM alone showed different changes such as disrupted lobular architecture with degenerating hepatocytes while others contained bi-nucleated hepatocytes, dilated congested central vein, and mononuclear cellular infiltration were appeared (Figure. 3). Also, Figure 4 found necrosis areas, dilated blood vessels, marked inflammatory cell infiltration, and corrugated central vein surrounded by fibrotic area. Moreover, the portal triad (Figure. 5) showed dilated congested and thickening wall of portal vein surrounded by fibrotic area, pyknotic nuclei in some hepatic cells, infiltration of inflammatory cells, degeneration, and disorganization of hepatocytes. On the other hand, PCM treated liver showed degeneration, and bi-nucleated hepatocytes, congestion of blood sinusoids, and pyknotic nuclei in some hepatic cells, necrosis with marked inflammatory cell infiltration, and areas of hemorrhage (Figure. 6).

The liver section of animals treated with VC for 7 days then given PCM for 7 days preserved the general architecture and lacked evidence of major morphological. Hepatocytes
degeneration appeared to be remarkably reduced and a few cellular infiltrations of both central vein when compared with the PCM group (Figure. 7). Microscopically, the liver of rats treated with VC + PCM for 14 days showed some alterations in liver architecture ranging from extensive dilated congested blood vessels with some inflammatory cell infiltration, degeneration of some hepatocytes, congestion of blood sinusoids, congested central vein, and increase of Kupffer cells (Figure. 8).

**Figure 1:** Photomicrograph of the liver section of control rats showing, lobular architecture: normal central vein (long arrow), and hepatocytes structures (thick arrow) (H & E, X400).

**Figure 2:** Photomicrograph of the liver section of treated rats by VC for 14 days showing, lobular architecture; normal central vein (arrow), and hepatocytes structures (H & E, X400).

**Figure 3:** Photomicrograph of the liver section of treated rats by PCM for 14 days showing, eroded area (long arrow), dilated congested central vein (star), and inflammatory cells infiltration (thick arrows) (H & E, X400).

**Figure 4:** Photomicrograph of the liver section of treated rats by PCM for 14 days showing, necrosis areas (double arrows), dilated blood vessels (long arrow), inflammatory cell infiltration (star), corrugated central vein (head arrow), surrounded by fibrotic area (thick arrow), and degenerating hepatocytes (short arrows) (H & E, X400).
Histopathologic changes in the liver tissues:

The changes in the histologic structure of the liver tissues were graded in (Table. 1) showed, reduction in the inflammatory cells infiltration, congestion, dilation, degeneration,
necrosis, and hemorrhage in the liver tissues in the protective group when compared with the PCM group.

Table 1: Histopathologic changes in the liver tissues.

<table>
<thead>
<tr>
<th>Histopathologic changes in liver tissues</th>
<th>NC</th>
<th>VC</th>
<th>PCM</th>
<th>PRO</th>
<th>VC+PCM</th>
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<tr>
<td>Inflammatory cell infiltration</td>
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<td>Hemorrhage</td>
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</table>

**(*) showing no changes, (+), (++), and (+++) indicating minimum, moderate, and maximum changes.

* NC = Normal control. PCM= Paracetamol treated group. VC= Vitamin C treated group. PRO =Protective group.

VC+PCM=Vitamin C + Paracetamol.

**Discussion:**

Paracetamol is metabolized in the liver by glucuronidation and sulfation pathways, an overdose triggers a saturation of these pathways resulting in a shift towards CYP450-mediated depletion of hepatic glutathione [20]. Downregulation of hepatic glutathione further elicits mitochondrial dysfunction, generation of ROS and tissue necrosis [21]. Additionally, PCM can produced hepatic toxicity and renal damage at lower doses [22].

The study showed that the liver injury by PCM alone had marked hepatocellular degeneration while others contained bi-nucleated hepatocytes, dilated congested central vein, mononuclear cellular infiltration, necrosis areas, dilated congested and thickening wall of portal vein surrounded by fibrotic area, pyknotic nuclei in some hepatic cells, dilated congested blood vessels with inflammatory cell infiltration, and areas of hemorrhage. These morphological findings were in accordance with the results of [4], [23], [24] who found that the administration of PCM produced marked many histopathological changes in the hepatic tissues.

Hassanin et al. [25] and Hassan et al. [24] suggested that liver damage is not due to the paracetamol itself but to its toxic metabolite of paracetamol, most of PCM is converted to
sulfate and glucuronide, and the rest, converted to NAPQI which is produced by cytochrome
P-450 enzymes in the liver. NAPQI is a reactive chemical and detoxified by enzyme
glutathione peroxidase. Excessive production of NAPQI causes liver damage as a result of
decline in the concentration of glutathione [26], [27]. At the time of glutathione deficiency
and presence of NAPQI, the production of free radicals like hydrogen peroxide, superoxide
and hydroxyl radicals has been increased and leads to oxidative stress [28]. The
degeneration of liver tissues and cellular metabolism could be degenerative changes in the
cytoplasm, where the integrity of the cytoplasm is quite important for the maintenance of
cellular vital functions. Also, structural changes in the nucleus and endoplasmic reticulum
which play an important role in the biosynthesis of glycoconjugates could probably lead to
impairment in glycolization mechanisms. Moreover, the integrity of the mitochondrial
membrane is important for the maintenance of vital functions and determination of the
apoptotic process. So these degenerations in mitochondrial membranes and cristae structure
could have negatively affect oxidative metabolism and vital balance limits of the cell [29].

On the other hand, administration of VC before PCM showed a marked reduction in
the histological changes. Hepatocytes degeneration appeared to be remarkably reduced and a
few cellular infiltrations of both central vein and portal triads when compared with the PCM
group. These results are in agreement with [6], [25] who reported the administration of VC
before paracetamol showed a marked reduction in the histopathological changes in the liver
tissue. They added that the mechanism by which VC prevents liver injury appears to involve
the destruction of reactive PCM metabolites which is associated with a sparing action on
liver toxicity. VC, as an antioxidant agent, may have inhibited the chain reactions of PCM-
generated free radicals or scavenged the reactive free radicals before reaching their hepatic
targets [15]. Studies have shown VC to be a potent antioxidant that mediates its antioxidant
effect by scavenging free ROS [30].

The ability of VC to trap free radicals, protect bio membranes from peroxide damage,
and effectively scavenge reactive oxygen species as reported by Sminorff and Wheeler [31]
may be suggestive of its effect exhibited in the treatment groups. Sabiu et al. [6] has also
reported VC as an excellent electron donor to free radicals which subsequently quench their
deleterious activity on cellular macromolecules, thus playing a role in antioxidant
mechanism. Many studies indicate the ability of VC to protect the hepatic cell from oxidative damage and lipid peroxidation which mediated by oxygen–free radicals. Also, the protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of hepatocytes [32]. Hassan et al. [24] detected that the VC administered orally caused some protective results with inflammatory cells and congested central veins in liver tissues. Nevertheless, the combination of VC with PCM for 14 days showed some alterations in liver architecture ranging from extensive dilated congested blood vessels with some inflammatory cell infiltration, degeneration of some hepatocytes, congestion of blood sinusoids, congested central vein and increase of kupffer cells, congested and thickening wall of portal vein surrounded by fibrotic area, and pyknotic nuclei in some hepatic cells. This submission is in conformity with the report of Hamza and Al-Harbi [33] who showed that PCM could in severe cases lead to liver failure in experimental animals and humans when taken in overdose for a long time.

Histopathologic assessments of the experimental parameters consistent with histological findings of the liver tissues were showed that the VC to be protective against PCM-induced hepatic damage.

**Conclusion:**

The present findings clearly demonstrate that paracetamol is capable of inducing histopathological changes in the liver tissues of the experimental rats. Besides, that VC has a protective effect against hepatotoxicity PCM-induced in male rats. In the near future, VC may be found useful as prophylactic agent against drug-induced hepatotoxicity.
References


