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Original Article

Hepatoprotective Effect of Vitamin B12 in Acetaminophen Induce Hepatotoxicity in Male Rats

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Abstract

Acetaminophen is a pharmaceutical synthesized non-opioid analgesic that belongs to the "aniline analgesics" class of medicine. Because it lacks a significant anti-inflammatory effect, it is not classified as a non-steroidal anti-inflammatory therapeutic medication (NSAID). As an over-the-counter pain reliever and antipyretic, Acetaminophen is the active metabolite of phenacetin and acetanilide, but it is less toxic than either precursor. According to some medical studies, Acetaminophen toxicity can be treated with vitamin B12. Acetaminophen-poisoned Male Wister rats were the subject model of the current study, which examines the effects of vitamin B12 on their hepatic health. There were three groups of animals: Acetaminophen treated animals (750 ml/kg), vitamin B12-treated animals (0.63 g/kg), and a control group that received distilled water (750 ml/kg). All animals were given oral medication for seven days. On the seventh day, the animal was sacrificed. Plasma levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Glutathione (GSH), total antioxidant capacity (TAC), Caspase3, Malondialdehyde (MDA), Interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-) were measured in the cardiac blood samples. Vitamin B12 lowers liver enzyme levels in the blood, increases overall antioxidant levels, and compensates for tissue glutathione deficiency while lowering serum elevations. TNF-α and interleukin-6 levels are also reduced by caspase3. Acetaminophen-induced hepatic necrosis and inflammatory cell infiltration were both considerably reduced by vitamin B12 supplementation. According to this study, vitamin B12 was found to have a protective effect against acetaminophen-induced hepatotoxicity.

Keywords: Acetaminophen, Hepatotoxicity, Vitamin B12

1. Introduction

Acetaminophen is a pharmaceutical synthesized non-opioid analgesic that belongs to the "aniline analgesics" class of medicine. Because it lacks a significant anti-inflammatory effect, it is not classified as a non-steroidal anti-inflammatory therapeutic medication (NSAID). Acetaminophen is an over-the-counter analgesic and antipyretic that is the active metabolite of phenacetin and acetanilide, but it is less toxic than either precursor (1). The Medicines and Healthcare Products Regulatory Agency (MHRA) has approved an oral paracetamol dose of 325 mg to 1 g every 4 to 6 hours for adults (maximum daily dose of 4 g per day) (2). According to recent research, at least 6% of filled prescriptions for Acetaminophen alone or Acetaminophen in conjunction with opioids surpassed 4000 mg per day, which is cause for serious worry (3, 4). At a mortality rate of 0.4%, there are about 300 overdose deaths each year. A rising number of studies indicate that very modest doses of APAP can cause acute liver injury and

liver failure, even though the most severe ingestions that result in hepatic failure typically exceed 150 mg/kg (5).

Antioxidants are chemicals that shield the body from permanent harm. Oxidative stress occurs when the body's defensive mechanisms fail to remove the oxidative consequences of free radicals when they are present in the body. This is because free radicals activate it and reduce the activity of effective oxygen species (6). As a co-antioxidant, antioxidants can impact biological systems through electron donation and metal ion chelation (7). Known as the red vitamin, vitamin B12 was discovered in 1926 and is also known as cobalamin or the Antianemique Vitamin (B12). Vitamin B12 and other B-complex vitamins are essential for energy metabolism and other cellular processes. In light of this, it differs from the other B-complex vitamins in several ways (8). Vitamin B12 is one of the most physiologically relevant antioxidants due to its chain-breaking mechanism and electron donation method. Reactive oxygen and nitrogen species that are secondary antioxidants can be removed by quenching a chain-initiating catalyst (9). Because APAP-induced liver damage is the most common cause of drug-induced liver failure and is believed to be caused by an increase in intracellular oxidative stress in hepatocytes, it was determined that this study would focus on the hepatoprotective effects of Vitamin B12 on APAP-induced liver damage.

2. Materials and Methods

2.1. Chemicals

We purchased a 500 mg acetaminophen tablet from the British company GSK. BioMerieux, France, supplied the reagent kits for the detection of transaminases. Elisa kits for determining total antioxidant capacity were bought from BT LAB, China, for tissue malondialdehyde, glutathione (GSH), caspase 3, and total antioxidant capacity (TAC). Methods for each diagnostic kit's work were followed precisely.

2.2. Experimental Animals

Twenty-one mature and healthy adult albino male rats with an age range between (10-13) weeks and weight from (180-200g) were obtained from an animal house of the College of Science/ University of Kufa. The animals were housed in plastic caged. The cage was embedded with wooden shelves below natural (12hrs.) light and (12hrs.) dark cycle at room temperature (23–25°C), and humidity was kept at (60–65%), and they were allowed to drink tap water and given standard chow diet ad libitum. The rats were kept in the animal house for acclimation to laboratory conditions for two weeks before they were used for the experiment to stabilize the stress caused by the change in their environment. 21 male rats were randomly divided into three groups (7 animals/group) and treated for 7 days.

•Group one: Rats administrated distill water 1 ml/kg/day orally

•Group two: Rats administrated only distill water and Acetaminophen at a dose of 750 ml/kg/day orally for 7 days.

•Group three: Rats administrated Vitamin B12 at a dose of 0.63 µg/kg /day orally for 7 days + Acetaminophen at a dose of 750 ml/kg/day orally on the seventh day. All animals were sacrificed on the eighth day.

2.3. Tissue Sampling for Histopathology

For histopathological exams, the apical portion was saved and fixed in 10% neutral formalin, then embedded in paraffin block and sliced into parts with a thickness of 5µM using a microtome. Light microscopy investigated the sections stained with hematoxylin and eosin blue12.

2.4. Statistical Analysis

Statistical analysis was carried out using SPSS version 27. Continuous variables were presented as (Means±SD). ANOVA test was used to compare means between three groups or more. A *P*-value of ≤ 0.05 was considered significant.

3. Results

3.1. Effects of Vitamin B12 on Liver Function Test

To see how Vitamin B12 administration compared to Acetaminophen and a control group affected liver function tests, biochemical markers of stress,

inflammation, and apoptosis, see figure 1A considerable increase in liver enzymes serum alanine aminotransferase and aspartate aminotransferase was seen following acetaminophen administration (750 ml/kg orally). As a result of treatment with Vitamin B12, these enzymes were reduced significantly and returned to normal levels (Table 1). Antioxidant depletion (GSH level) and lipid peroxidation (MDA level) in liver tissue caused by Acetaminophen was greatly improved by Vitamin B12 treatment, as were levels of total antioxidant capacity (TAC) and anti-inflammatory markers (TNF-α and IL-6).

3.2. Effects of Vitamin B12 on Liver Histopathology

treatment with vitamin B12 only led to minor liver deterioration, while kupffer cell growth and no necrosis were observed in the positive control group (Figure 2).

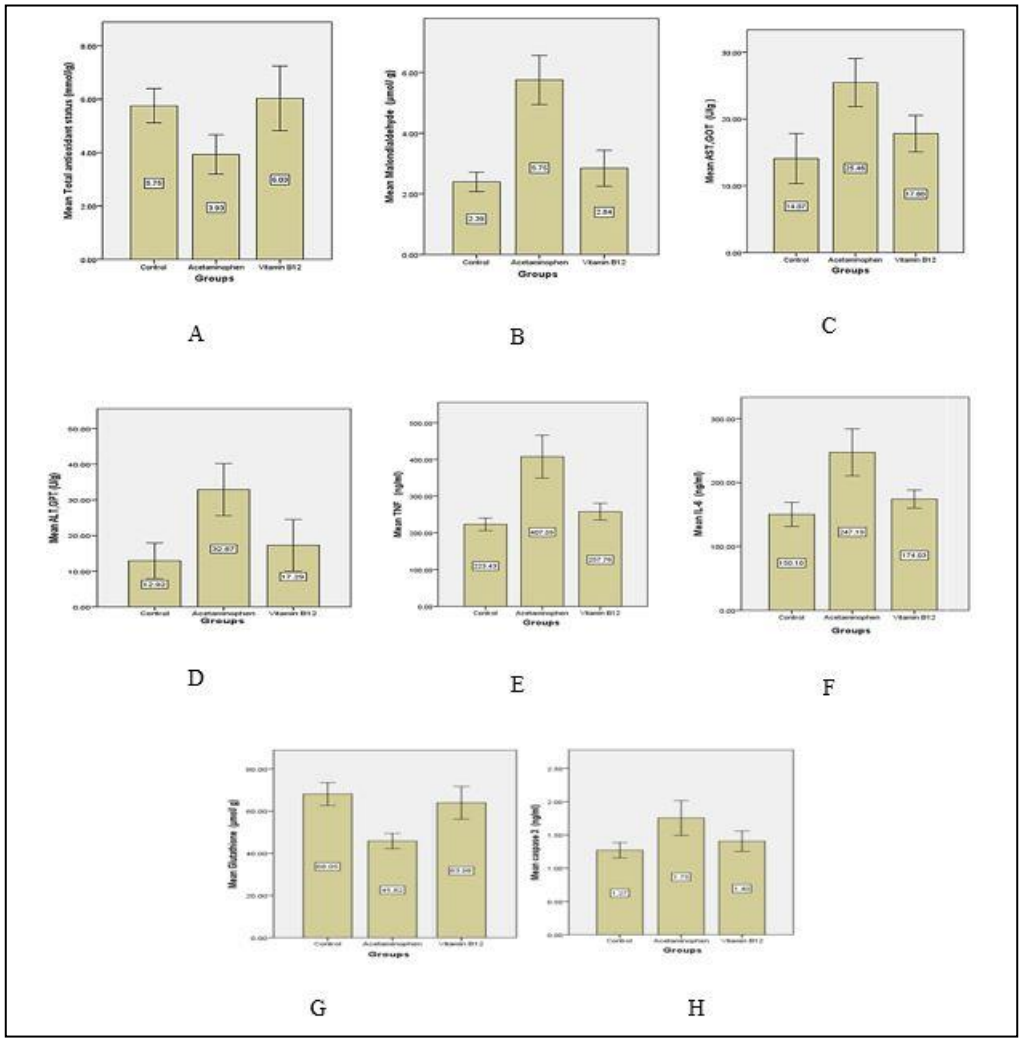

Figure 1. Effects of Vitamin B12 on liver function tests, biochemical, oxidative stress, inflammatory, and apoptosis parameter compared to Acetaminophen and control group (A: total antioxidant capacity, B: malondialdehyde, C: Aspartate aminotransferase (AST), D: Alanine aminotransferase (ALT), E:(Tumor necrosis factor- alpha (TNF-α), F: Interleukin-6 (IL-6), G: Glutathione, H: Caspase 3)

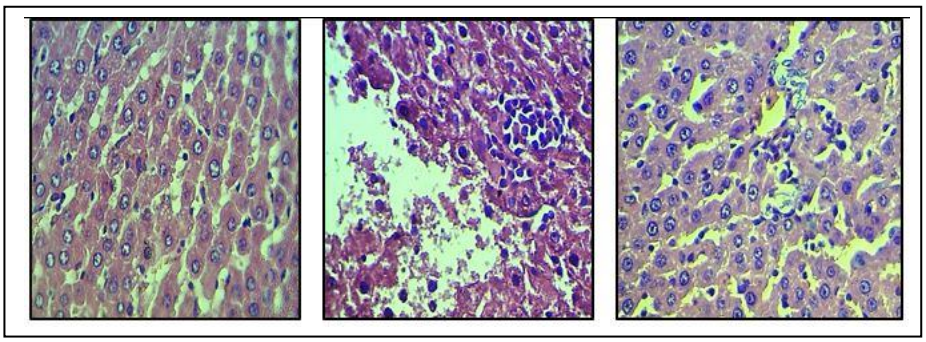

Figure 2. Section of liver of albino male rat of study groups (**A:** Normal group, **B:** Acetaminophen group, **C:** Vitamin B12 group) on day 8 of the experiment. 400X, H&E

Table 1. Represents the Histopathological changes that were observed, representing (0) no symptoms, (1) mild symptoms, (2) moderate symptoms, (3) severe symptoms (4) acute symptoms

Group 3	Group 2	Group 1	Groups Histopathological changes	
1	2	0	Hydropic degeneration	Hepatocellular changes
0	3	0	necrosis	
0	2	0	Apoptosis	
2	0	0	Mitotic figure	
1	3	0	Congestion of the central vein	
2	3	0	Dilation of the sinusoids	
0	2	0	Portal	Inflammation
1	3	0	lobular	
2	1	1	kupffer cells proliferation	
0	3	0	bile duct injury	Bile ducts
0	2	0	bile duct hyperplasia	
0	2	0	portal	Fibrosis
0	1	0	Septal	
0	0	0	perisinusoidal	

In this work, the histological analysis of male rat liver sections from the control group reveals typical histological features. The results are shown in figure 2.

Histopathological examination of liver male rat section of group 2 treated with Acetaminophen at a dose (750 ml/kg/day) showed activation of kupffer cells, irregular and enlarged portal tract, necrosis with the appearance of newly formed bile ductules as shown in figure 2 compared with the control group. The histopathological score of rat liver group 2 are shown in table 1. The results showed that there was damage noted in hydropic degeneration, congestion of the central vein, no damage in the mitotic figure, and no damage in perisinusoidal fibrosis. Mild toxicity was seen as kupffer cell proliferation. Moderate toxicity was seen as apoptosis and portal Inflammation; severe toxicity was seen in congestion of the central vein, lobular inflammation and bile ducts, necrosis, and dilation of the sinusoids. Histopathological examination of liver male rat section of group 3 treated with Vitamin B12 at a dose (0.63 µg/kg /day) shows dilation of the sinusoids with mild mononuclear cells infiltration and increase in the number of kupffer cells figure 2 compared with the control group. The histopathological score of rat liver group 3 are shown in table 1. The results showed no fibrosis, congestion of the central vein, no, or bile duct injury or necrosis, and no congestion of the central vein. There were no apoptotic symptoms and only minor indications of hydropic degeneration, but there were moderate symptoms of inflammation and dilatation of the sinusoids, and a mitotic pattern was observed.

4. Discussion

Even though Acetaminophen has long been considered safe, the overall safety of Acetaminophen at therapeutic, licensed levels has lately been questioned because multiple studies have shown alanine aminotransferase increases in asymptomatic patients (12, 13). Patients with hypertension and migraines regularly and continuously use Accutane or myocardial infarction to lessen headaches or ill-defined discomfort related to these diseases (11). All rats who received APAP or distilled water for seven days did not die. Antioxidants are thought to counteract the detrimental effects of Reactive Oxygen species and prevent or treat oxidative stress-related diseases. Since certain liver illnesses can benefit from antioxidant therapy, it has been considered an option.

4.1 Effect of Acetaminophen on Oxidative Stress (MDA, TAC, GSH) in Liver Tissue

MDA is a marker of oxidative stress; it is a reactive aldehyde, one of the final products of polyunsaturated fatty acids peroxidation in the cells; therefore, it increases free radical formation, resulting in increased production of MDA (11).

Acetaminophen-induced hepatotoxicity by increasing oxidative stress and increasing free radical formation. So, MDA is one of the indicator markers used in the current study. After 7 days of Acetaminophen administration, MDA increased significantly in the Acetaminophen group compared with the control group, indicating high oxidative stress and hepatotoxicity. This agrees with a study by Damiano, Muscariello (10), .whereas pretreatment with vitamin B12 significantly inhibited such an increase.

Total antioxidant capacity (TAC) is an important marker in hepatoprotective studies as it determines the cumulative effect of all the antioxidants in blood and body fluids. The human body generally has an antioxidant system that plays an important role in suppressing ROS overproduction. This protects the cells from oxidative stress, which is one of the APAP-induced hepatotoxicity (14). TAC level in the APAP group decreases significantly compared with the vitamin B12 group due to the effect of APAP to increase oxidative stress and ROS formation; this agrees with the study by (15).

4.2 Effect of Acetaminophen on Serum Liver Function Tests (ALT and AST)

Acetaminophen treatment increased the serum levels of the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are found in the cytosol and released into the blood after liver damage, significantly compared with the control group's values. This increase is thought to be due to damaged liver cells. Sedaghattalab, Razazan (16), found that, in the APAP group, the increase in AST and ALT was more pronounced. Preventing in vivo hepatic blood levels of liver enzymes caused by an APAP overdose can be achieved by pretreatment with vitamins B12 (17).

4.3 Effect of Acetaminophen on Serum Inflammatory Parameters (TNF -α and IL-6)

TNF- and IL-6 are macrophage/monocyte-produced pro-inflammatory cytokines (18). An increase in the release of TNF-α and TNF-α in the APAP caused liver injury linked to pathological liver changes (19). TNF-α and IL-6 concentrations in rats exposed to APAP livers were significantly elevated. In the blood, APAP elicited an inflammatory response. On the other hand, rats treated with vitamin B12 had much lower levels of TNF-α and IL-6 in their livers. As a result of this protective effect compared to the APAP group, the levels of hepatic TNF-α and IL-6 dramatically decreased, as were the infiltrating inflammatory cells in histological liver sections. Han, Zhang (20) findings were consistent with this study's.

4.4 Effect of Acetaminophen on Apoptosis Parameter (Caspase 3)

Anthracycline's main apoptotic route, Caspase-3, functions by compromising the integrity of the mitochondrial membrane, making it an apoptosis indicator (18). The Mitochondrial Permeability Transition (MPT) is an important coordinating

event in the apoptotic process, which involves a rapid rise in mitochondrial membrane permeability. MPT triggers the mitochondrial release of cytochrome C, which then activates effector caspases to cause the development of a DNA ladder (21). Caspase-3 levels in the vitamin B12-treated group were lower than those in the APAP-treated group, APAP treatment raised the levels of caspase-3. B12 is an essential vitamin for the survival and death of cells in the hepatocellular matrix because it restores mitochondrial activity and regulates intracellular redox status, which is critical for hepatocyte survival and death (20). Fission and destruction of injured mitochondria are possible due to the pathogenic ROS produced by healthy mitochondria that drive biological activities, prevent cell death, and sustain mitochondrial function. The mitochondrial fission events are regulated by oxidative stress (22).

Vitamin B12 is protective in Acetaminophen-induced hepatotoxicity rats.

Authors' Contribution

Study concept and design: R. A. M.
Acquisition of data: Q. J. F.
Analysis and interpretation of data: R. A. M.
Drafting of the manuscript: Q. J. F.
Critical revision of the manuscript for important intellectual content: Q. J. F.
Statistical analysis: R. A. M.
Administrative, technical, and material support: R. A. M.

Ethics

The animal study was approved by the ethics committee of the University of Kufa, Kufa, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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