

New Animal Model of Induced Acute Hepatic Injury by Acetaminophen and Vitamin D3 Protective Effect

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Abstract

Background: Drug-induced liver injury is of great interest especially drugs that broadly used like acetaminophen. **Objective:** To assess new model of acetaminophen-induced acute hepatic injury and Vitamin D3 effectiveness. **Materials and Methods:** Eighteen male rabbits allocated into three groups six rabbits for each. Group 1 receives acetaminophen to induce acute hepatic injury and Group 2 receive single injection of Vitamin D3 before induction of hepatic injury, and group 3 was control. After 24 h from three-spaced injection of acetaminophen sample of blood taken to measure serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and albumin. **Results:** Successful model of acetaminophen-induced hepatic injury achieved by significant elevation ($P \leq 0.05$) of ALT and AST of Group 1 and 2 compared with control. No significance variation observed ($P \geq 0.05$) between treatment Group 1 and 2 in liver function enzymes level. **Conclusion:** A new easy model of acetaminophen-induced acute hepatic injury effectively obtained. Although no statistical significant Vitamin D3 lower the levels of ALT and AST.

Keywords: Acetaminophen, hepatic injury, Vitamin D3

INTRODUCTION

Liver is responsible for the detoxification of various substances including drugs and xenobiotic. Phase I and phase II enzymes play an important role in the metabolism and detoxification of drugs and xenobiotics.^[1] Intake of drugs, their metabolism and removal make a condition affect dynamic equilibrium lead to shift toward free radicals generation and oxidative stress, which impair normal liver function.^[2]

Mechanisms involved in drug-induced liver impairment include exhaustion of glutathione reduced form, ability to cause covalent bond with essential proteins, lipids, and/or nucleic acids, encouraging lipid peroxidation besides to immunity response induction. All these mechanisms could induce consequence of events in hepatocytes organelles causing traumas that deteriorate normal hepatocytes functions, damage, and death.^[2,3]

Stress on hepatocytes leads to continuous inflammation similarly stimulates hepatic stellate cells, myofibroblasts, and fibroblasts principal to liver fibrosis.^[4] Acetaminophen is one of these drugs that have the ability to impair liver function when used in large doses leading to acute liver failure.

Acetaminophen in therapeutic daily dose metabolized by liver to Nacetyl-p-benzoquinoneimine (NABQI) which regarded harmless when further conjugated to glutathione. In case of overdose, hepatic glutathione supplement will be depleted and NABQI will oxidize thiol group of hepatocellular enzyme leading to cell death.^[5,6]

Preservation of normal hepatocytes function from drug effects is vital goal and using of Vitamins and herbal products aimed to provide liver protection.^[7] Vitamin D is one of fat-soluble vitamins and regards as a hormone.^[8] Vitamin D exists in two biological form Vitamin D2 (ergocalciferol) and D3 (cholecalciferol).^[9] The main source of Vitamin D is sun exposure and food. Exposure to sun ultraviolet of wavelength range (290–320 nm) leads to convert 7-dehydrocholesterol in the skin to proVitamin D3 while Vitamin D2 source is food such as plants and some fish. Both forms of Vitamin D

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transported to the liver where they hydroxylated to 25(OH)D (the inactive form of Vitamin D) and then to the kidneys where they undergo another hydroxylation by the enzyme 1- α hydroxylase to 1, 25(OH)D, the active form.^[10,11]

Vitamin D (active form) act through binding to special nucleic receptors called Vitamin D receptors (VDR) presented in cells of most tissues. Binding of Vitamin D to its receptor will lead to a series of biological function implicated to Vitamin D action. According to that, Vitamin D is hormone rather than vitamin.^[12,13] The classical role of Vitamin D and its receptors is regulation of calcium absorption from intestine and maintain bone and calcium hemostasis by control duodenum calcium absorption and renal calcium reabsorption.^[13]

Vitamin D found to have great role in the protection of hepatocytes and maintain normal liver function since hepatocytes express VDR. A study by Hochrath *et al.*^[14] show that a Vitamin D-sufficient diet ameliorates chronic liver injury through decrease collagen accumulation and fibrogenesis. Another study done by Papapostoli *et al.*^[15] declare that patients with hepatic steatosis and inadequate Vitamin D level when received Vitamin D replacement therapy correct their state significantly. Mechanisms by which Vitamin D protect liver are many. Vitamin D has the ability to attenuate inflammatory process by multiple mechanisms such as its ability to reduce T helper 1 cell response and shifting toward T helper 2 response with reduction of inflammatory mediators such as tumor necrosis factor alpha, interferon gamma, and interleukins 2 (IL-2), 12.17, and IL-21. In addition to increase production of anti-inflammatory cytokines such as IL-10,23 and other cytokines. Moreover, VDR is expressed on macrophages and other dendritic cells in addition to the ability of these cells to produce active Vitamin D.^[16]

The aim of this study was to assess new model of acetaminophen-induced acute hepatic injury and Vitamin D3 effectiveness.

MATERIAL AND METHODS

Animals

Eighteen local male rabbits obtained from local market weighing 1100–1300 g. Included in study design all of them healthy and free from infection. The rabbits housed in animal house related to the college of medicine, University of Babylon retained in standard cages with free access to stander pellet food and tap water. A temperature of 22°C \pm 1°C with a 6 am–6 pm light phase provided to ensure standard circumstances for all animals. Rabbits supplied with numbers assigned to computer program to allow random grouping. Then, rabbits divided into three groups randomly.

Drugs

1. Acetaminophen (Apmol) 500 mg/5 ml, suitable for intramuscular and intravenous injection (Ajanta. Pharma limited, India).
2. Vitamin D3 (Cholecalciferol) of dose strength 300.000 I.

U./1 ml suitable for injection intramuscularly (Dibase, Abiogen PHARMA, Italy).

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Study design

Rabbits randomly allocated into three groups as: Group 1: Six rabbits supplied with acetaminophen to induce acute hepatic injury. Group 2: Six rabbits take single intramuscular injection of Vitamin D3 at dose 100.000 IU equal to 0.33 ml at about 24 h before induction of acute hepatic injury.

Group 3: Six rabbits set as control with no management.

Induction of acute hepatotoxicity

Acetaminophen-induced acute hepatic injury model established in this study was according to^[17] but with alteration in dose strength and route so that three doses of acetaminophen (500 mg) injected intramuscularly in spaced manner.

At the beginning 500 mg of acetaminophen injected at 9 am, then a second 500 mg injected after 9 h from the first dose. At the next day (24 h from the initial dose), the third 500 mg of acetaminophen administered [Table 1].

Later, a sample of blood about 3–5 ml collected from marginal ear vein after 24 h from the last dose. Blood transferred into a nonheparinized tube and let for 30 min for clotting. Followed by centrifugation at 3000 rpm for 20 min to separate serum, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and albumin levels measured.

Statistical analysis

To compare liver enzymes levels between groups, independent-samples Student's *t*-test used at level of statistical significance $P \leq 0.05$ by SPSS version 22 (IBM). Data expressed by means \pm standard deviation.

RESULTS

Rabbit model of acetaminophen-induced acute hepatic injury positively achieved with no mortality. Statistical analysis of the ALT and AST values of Group 1 and 2, show significant elevation ($P \leq 0.05$) when compared with control (Group 3), while ALP and albumin values mean display no significant changes ($P \geq 0.05$) as appear in Table 2.

On the other hand, significance variation was not observed ($P \geq 0.05$) between treatment Group 1 and 2 by comparing ALT

Table 1: Acetaminophen-induced acute hepatotoxicity timetable

1 st acetaminophen dose 500 mg	2 nd acetaminophen dose 500 mg	3 rd acetaminophen dose 500 mg	Blood collection
0 h	9 h	24 h	48 h

Table 2: Liver function tests changes between Group 1 and 2 compared with control expressed by mean±standard deviation

Liver function tests	Group 1	Group 2	Group 3 (control)
ALT (u/l)	161.333±32.254*	145.0±6.082*	67.666±22.722
AST (u/l)	89.00±19.052*	117.00±20.421*	28.666±2.0816
ALP (u/l)	28.333±22.279	50.666±18.823	90.666±43.661
Albumin (g/l)	2.933±0.378	3.266±0.153	3.333±0.11547

* $P \leq 0.05$. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

Table 3: Liver function test changes between Group 1 and 2 expressed by mean±standard deviation

Tests	Group 1	Group 2
ALT (u/l)	161.333±32.254	145.0±6.082
AST (u/l)	89.00±19.052	117.00±20.421
ALP (u/l)	28.333±22.279	50.666±18.823
Albumin (g/l)	2.933±0.378	3.266±0.153

$P \geq 0.05$. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

levels and AST levels; however, levels were lower in Vitamin D3-treated group regarding ALT level as presented in Table 3.

DISCUSSION

Liver is widely subjected to cellular injury due to drug intake for prevention or treatment since liver is an organ of biotransformation, this process leads to metabolites affect hepatocyte by inactivate or accelerate enzymatic function and exhaustion of conjugate store.

Acetaminophen is analgesic and antipyretic drug usually used for mild and moderate pain. The reason of Acetaminophen choice in this study was due to availability over the counter that render patients to use with no limit whenever need. The major adverse effect of that drug is acute liver failure, especially at a dose exceeding 4 g/day. Exposure to that dose could be suicidal or accidental for pain or fever.^[18]

Acute hepatic injury model established by three-spaced acetaminophen doses according to Francavilla, who used dog model for fulminant hepatic failure by acetaminophen in three-spaced doses of about 200 mg/kg subcutaneously. However, our model of acute hepatic failure established with higher dose, intramuscular route, and rabbit species. This model proves its efficiency and success depending on liver enzymes result and absence of mortality Table 2.

Reaching to that result provide a model easy for the researchers to obtain with preserving animals life and cost. Many models of acetaminophen-induced acute liver injury available from different studies using large doses and associated with animals mortality due to causes rather than hepatic failure like elevation of mitochondrial oxidative stress that provoke cell death in other organs.^[19]

Liver function tests are important indicator of hepatocytes function, especially ALT and AST enzymes. These enzymes usually located intracellular but due to injury leakage happened lead to increase their serum level especially for ALT enzyme which, mainly exist in hepatocytes. Study results declare significant raise in ALT and AST levels of treatment groups as compared with control. This finding indicate achievements of new acute hepatic injury model by acetaminophen which recognized for its ability to cause necrosis and apoptosis in hepatocytes organelles in large doses.^[20] This effect is due to depletion of hepatic store of glutathione group leads to bind of acetaminophen metabolites to specific proteins in hepatocytes organelles cause increase of hepatic enzymes in serum.^[21]

On the other hand, no statistical significance observed between Vitamin D3 treatment group (Group 2) and acetaminophen-induced hepatic injury group (Group 1) regarding hepatic serum enzymes level even though levels of ALT enzyme appear lower in Vitamin D3 treatment group. This finding could explained by smaller animal sample or in adequate amount of Vitamin D3 supplements, which require further evaluation of sample size in accordance with Vitamin D3 dose. Levels of AST appear higher in Group 2 as compared with Group 1 this could be explained by muscle injury that result from intramuscular injection of Vitamin D3 and acetaminophen in Group 2 rabbits while Group 1 injected with acetaminophen only. Vitamin D3 documented to ameliorate drug-induced injury in pancreas and renal system; furthermore, it diminutions oxidative stresses and hepatic steatosis.^[22]

ALP and albumin levels show no significance differences between study groups this reach agreement with Chun *et al.*^[23] who found fewer changes in ALP level in acetaminophen-induced hepatic failure.

CONCLUSION

A new model of acetaminophen-induced acute liver injury effectively proven with preservation of cost and animals.

In spite of no statistical significant Vitamin D3 have protective effect in accordance to liver function tests; that require additional studies to prove.

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Conflicts of interest

There are no conflicts of interest.

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