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The Effect of Short-term Periodic Fasting on Acetaminophen-induced Liver Injury in Mice

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ABSTRACT

Introduction: In many cultures, fasting is recommended for health protection and promotion. However, few studies have been focused on the effects of fasting on organ function and resistance to toxic agents (e.g., drugs). The present study aimed to investigate the effects of short-term periodic fasting on the hepatotoxic effects induced by acetaminophen in mice.

Methods: This experimental study was conducted on female BALB/c mice to assess the effects of short-term periodic fasting (three consecutive days every two weeks for 10weeks) on the serum levels of aspartate transaminase (AST) and alanine amino transferase (ALT)and hepatotoxic effects induced by acetaminophen. After 10weeksof periodic fasting, the mice were administered with 500 mg/kg of acetaminophen via intra peritoneal injection. After 24 hours, the AST and ALT levels were measured, and the mice were sacrificed to evaluate their liver injury severity using the pathological method as the gold standard.

Results: The AST and ALT enzymes increased in the control group (P=0.0098 and P=0.0004, respectively; Mann-Whitney U test), which was associated with high-grade liver injury (P=0.001; Fisher's exact test). In contrast, the fasting mice had slight changes in the levels of AST and ALT enzymes associated with low-grade liver injury.

Conclusion: Acetaminophen is a common cause of drug-induced liver injury. According to the results of the study, fasting could protect important organs (e.g., liver) against the toxic effects of drugs. Further investigations in this regard could provide insight into human states.

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Introduction

Fasting is an intense mode of dietary restriction, which involves the avoidance of all foods (except water) with intervening periods of normal food consumption. Depending on the duration, fasting could be classified as intermittent fasting (≥16 hours of fasting/day or 48 hours of fasting/week) and periodic fasting (minimum of three days of fasting/two or more weeks)(1).Fasting not only decreases blood glucose levels and growth factor signaling, but it also activates the stress resistance pathways, thereby leading to the modulation of cell growth, energy metabolism, and protection against oxidative stress, inflammation, and cell death(1, 2).

According to Longo and Mattson, intermittent fasting in rodents could exert protective effects against cancer, cardiovascular diseases, diabetes, and neurodegeneration(2). In another study, Brandhorst et al. demonstrated that prolonged fasting (two or more days of dietary restriction followed by at least seven days of normal diet)could promote cognitive performance, decrease inflammation and cancer incidence, and extend the health span and multisystem regeneration of mice (3).

Recent data suggest that prolonged fasting plays a key role in the protection of healthy cells and

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organs against toxicity (4, 5).According to the previous studies on mice, caloric restriction could attenuate the lethal effects of hepatotoxins such as thioacetamide(6) and bleomycin(7).Acetaminophen (APAP) is used as an analgesic and antipyretic drug and has extensive application in medicine (8, 9).APAP is considered safe at therapeutic doses, while its overdose has been reported to be hepatotoxic(10).

The present study aimed to investigate the effect of short-term periodic fasting on liver injury following high-dose APAP administration.

Materials and Methods

Mice and Fasting Regimen

This experimental study was conducted on female BALB/c mice with short-term periodic fasting to investigate the toxic effects of high-dose APAP on the liver. At six weeks of age, the mice were housed in boxes (three animals per box) and randomly assigned to two groups of fasting and control. Both groups were fed *ad libitum*, each consisting of 20mice.

Specific pathogen-free conditions were maintained throughout the study. The animals were kept in a room within a 12-hour light/dark cycle at constant temperature and humidity. The fasting periods encompassed three consecutive days every two weeks. During these periods, the fasting mice only had access to water. Except for these three days, the animals had free access to adequate food and water. The fasting protocol continued for 10weeks.

Four days after the last fasting period, the animals in the fasting and control groups were administered with 500 mg/kg of APAP via intraperitoneal injection. After 24 hours, eight mice were randomly selected from each group to measure the serum levels of aspartate transaminase (AST) and alanine aminotransferase (ALT; U/L) using the commercial enzymatic kits of Biorex Fars Company and an automated analyzer (model: HITACHI 911). Following that, the animals were sacrificed to determine the severity of liver injury using the pathological method as the gold standard. For this purpose, macroscopic and microscopic changes were assessed. Also, liver samples were fixed in 10% formalin, and stained using the H&E method. The induced liver injury was examined based on special criteria, such as the parenchymal distortion of the liver and necrosis (Table 2-A).

Statistical Analysis

Data analysis was performed in SPSS version 17 using Mann-Whitney U test and Fisher's exact test to investigate the statistical differences between the control and fasting groups. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

Results

In normal mice, the serum levels of ALT and AST in plasma is ALT: 25-60 U/L; AST: 50-100 U/L; whereas the increased levels of these enzymes indicate liver injury. After APAP treatment, the AST levels increased in the control group(117.36±48.57U/L), while they remained within the normal range(61.13±21.75U/L) in the fasting group (P=0.0098). More significant results were obtained for the ALT levels., increased ALT (124.55±34.63) observed in the control group, and slightly increased levels (66.06±10.29) observed in the fasting group of mice (P=0.0004) (Table 1).

Table1. Measurement of Serum Aminotransferases (AST and ALT) in the Normal, Control and Fasting Groups of Mice (AST and ALT increased in control group compared to fasting group [P=0.0098 and P=0.0004, respectively]; Since variables had non-normal distribution, they were evaluated using Mann-Whitney U test.)

Serum aminotransferases	Normal mice	Control group	Fasting group	Mann-Whitney Test
AST (U/L)	50-100	117.36 ± 48.57	61.13 ± 21.75	P= 0.0098 df = 14 z = 2.99
ALT (U/L)	25-60	124.55 ± 34.63	66.06 ± 10.29	P = 0.0004 df = 14 z = 4.58

Significant differences were observed in the mean levels of ALT and AST in the control and fasting groups (P<0.05).The liver injury grading showed that all the mice in the control group (n=8) had high-grade injury(grades II, III, and IV), while the animals in the fasting group had low-grade injury (grades zero and I), and high-grade injury was only observed in one fasting

mouse(P=0.001)(Figure 1; Table 2-B).It is notable that the distribution of liver injury was not homogeneous in the study groups.

Table2. A) Histopathological Grading Based on Histopathological Studies; B) Liver Injury Grading in Control and Fasting Groups of Mice (High-grade liver injury in control group compared to fasting group [P=0.001]; Fisher's exact test used for qualitative variables.)

A	
Grading	**microscopic findings**
0	Normal hepatic architecture
I	Mild distortion of liver parenchymal architecture with minimal focal necrosis
II	Mild distortion of liver parenchymal architecture with several foci of liver necrosis
III	Moderate distortion of liver parenchymal architecture with multiple foci of liver necrosis
IV	Severe distortion of liver parenchymal architecture with multiple foci of liver necrosis

B			
Liver injury grade	**Control group**	**Fasting group**	**Fisher's Exact Test**
Low - grade (0, I)	0	7	P = 0.001
High - grade (II, III, IV)	8	1	

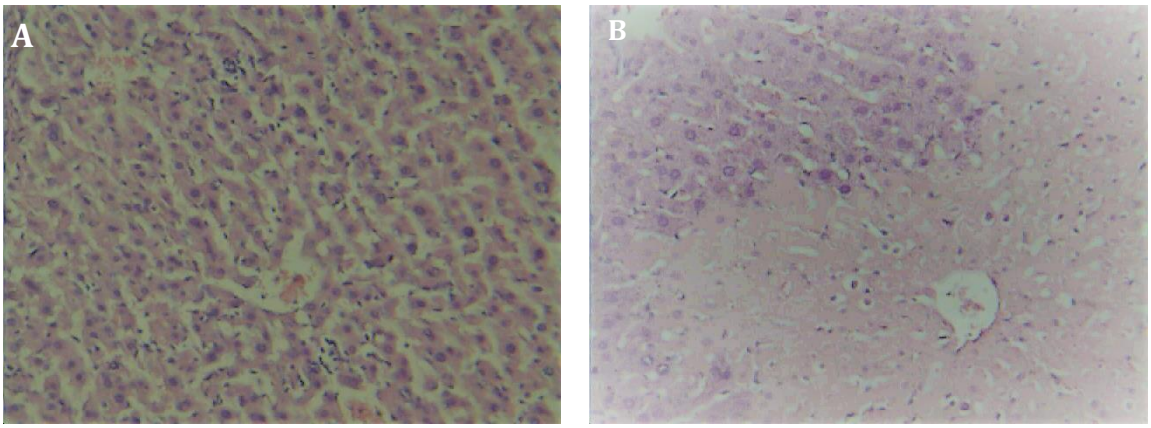

Figure 1. Photomicrograph of Histopathological Examination of Liver Samples (400x magnification; A: hepatocytes necrosis in control liver sample, B: The lack of any serious injury in periodic fasting liver sample.)

Discussion

APAP is a well-known hepatotoxin, which causes drug-induced liver injury in a dose-dependent manner and is also considered to be the most common cause of drug-induced liver injury (11). In addition, the side effects of APAP are regarded as a major public health concern (10). The liver is the main organ that metabolizes APAP extensively and rapidly, while the gut and kidneys are also involved in this process(12).The liver converts APAP into glucuronide and sulfate conjugates although the toxic metabolite of N-acetyl-p-benzoquinoneimine (NAPQI)is also produced in small amounts (13).NAPQI is conjugated with reduced glutathione in the liver and is exerted in the bile and urine. Since the liver has limited glucuronidation and sulfation capacity, the excessive consumption of APAP leads to the excessive production of NAPQI. Surplus NAPQI production is detoxified partially with glutathione, and the functional alteration of cell protein thiol via covalent binds with the remaining NAPQ eventually causes acute hepatic necrosis(13).The cellular events leading to liver injury following high-dose APAP consumption in mice model are associated with mitochondrial damage, oxidative stress, c-jun N-terminal kinase activation, and nuclear DNA fragmentation(14, 15).

Multiple factors potentially influence APAP-induced liver cell damage, including the hepatocyte responses to APAP metabolites(16),

pro-inflammatory cytokine cascades(17), heat shock proteins(18), peroxisome proliferator-activated receptors (19), and glutathione levels(20). Autophagy is a natural cellular mechanism, through which autophagosomes (double-membrane vesicles) engulf cytoplasmic cargos, subsequently fusing with lysosomes to form autolysosomes, where the unnecessary/dysfunctional components are degraded or recycled(21, 22). Stressful stimuli such as fasting (i.e., nutritional restriction), hypoxia, DNA damage, and cytotoxic agents may promote the autophagy phenomenon(23, 24). Furthermore, autophagy is an effective mechanism to adjust stressful conditions in most cells and organs, such as the liver and muscles (24, 25).

In the current research, we evaluated the impact of short-term periodic fasting (three consecutive days every two weeks for 10weeks) on the serum ALT and AST and liver injury following high-dose APAP administration (500 mg/kg) in female BALB/c mice. According to the obtained results, the intraperitoneal injection of APAP caused the AST and ALT serum levels to increase in the control group, while only slight changes were observed in the fasting group in this regard (P=0.0098 and P=0.0004, respectively).

In the present study pathological method was applied to determine the severity of the liver injury. According to the findings, high-dose APAP led to high-grade liver injury in the control group, while the fasting group of mice showed low-grade liver injury (P=0.001). This is in line with the results of the previous studies conducted on mice, which indicated that high-dose APAP may increase serum aminotransferases and leads to extensive liver injury (26-28). Furthermore, the comparison of the fasting group to the control group of mice indicated protection against APAP-induced liver injury which is consistent with the previous findings in this regard (16).

In congruence with our findings, Verweij et al. reported that fasting protects the liver against ischemia-reperfusion injury. Accordingly, fasting may lead to the up-regulation of antioxidant enzymes, such as superoxide dismutase 2, glutathione peroxidase 1, and glutathione reductase, as well as the stress response gene heme oxygenase 1(5). Interestingly, factors such as the inflammatory cascades, peroxisome proliferator-activated receptors, and glutathione levels have been shown to be remarkably influenced by calorie restriction (e.g., fasting)(19, 29-31), which attests to the protective role of fasting against drugs such as APAP(16).

Conclusion

According to the results, short-term periodic fasting could positively influence liver drug detoxification as the fasting mice had low-degree liver injury against high-dose APAP. Undoubtedly, further investigations are required on larger sample population in order to prove such beneficial effects.

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Conflicts of interest

The authors declare no conflict of interest.

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