

The Evaluation of the Possible Protective Effect of Honey on Acetoaminophen Induced Liver Damage in Mice

Ruaa F. Abbas

Ministry of Health, Advisor Office, Baghdad, Iraq

Kawther M. Ibrahim

Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad,

Sulake F. Alzubaidi

Department of Surgery and Obstetric, Collage of Veterinary Medicine, University of Babylon,

Abstract

In this study, eighteen male mice weighing (30 ± 2 gm) were used throughout the experimental study. The animals were divided randomly into three groups each group consisting of six animals. Group (I) mice acted as a control. Group (II) (intoxicated group) were received single dose of APAP (500mg kg⁻¹ body weight, i.p.). Group (III) mice were pre-treated with honey (600mg kg⁻¹ body weight, p.o.) for 8 successive days, and then the mice were intoxicated with APAP. Hepatotoxicity caused by APAP and the protective effect of honey in mice was studied by assessing serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels, and histopathological study of liver. In Group (II), APAP increased ALT and AST level significantly compared with control group. Pre-treatment of honey prior to APAP administration decreased the increment of ALT and AST level. The histopathological examination of the liver showed that honey reduced the incidence of hepatocyte damage induced by APAP. It was concluded that honey may attenuate the hepatotoxic effect of APAP and it could be used as an effective hepatoprotector against APAP-induced liver damage.

Key words: Honey, Hepatotoxicity, Acetaminophen.

الخلاصة

في هذه الدراسة تم استخدام 18 فأراً (ذكر) يزنون (30 ± 2 غم). قسمت الحيوانات عشوائياً الى ثلاثة مجاميع كل مجموعة تتكون من 6 حيوانات. المجموع الأولى عملت كمجموعة سيطرة. المجموعة الثانية (المجموعة المسممة) أستلمت جرعة مفردة من الأسيتوأمينوفين (500 ملغم/كغم من وزن الجسم داخل الصفاق). المجموعة الثالثة عولجت مسبقاً بالعسل بجرعة (600 ملغم/كغم من وزن الجسم عن طريق الفم) لثمانية أيام متتالية ومن ثم سممت الفئران بالأسيتوأمينوفين. تسمم الكبد الناتج من الأسيتوأمينوفين و تأثير العسل الحامي عند الفئران المختبرية درست عن طريق قياس أنزيم الألبانين أمينوترانفيريز و أنزيم الأسباريت أمينوترانفيريز ودراسة النسيج المرضي للكبد. في المجموعة الثانية تسبب الأسيتوأمينوفين في زيادة معنوية لأنزيم الألبانين أمينوترانفيريز و أنزيم الأسباريت أمينوترانفيريز مقارنة بمجموعة السيطرة. العلاج مسبقاً بالعسل قبل إعطاء الأسيتوأمينوفين تسبب بتقليل الزيادة الحاصلة في أنزيم الألبانين أمينوترانفيريز و أنزيم الأسباريت أمينوترانفيريز. فحص النسيج المرضي للكبد بين أن العسل قلل حدوث تلف خلايا الكبد المستحدث بواسطة الأسيتوأمينوفين. أستنتج من هذه التجربة أن العسل قد يضعف التأثير السام للكبد للأسيتوأمينوفين ويمكن أن يستعمل كحام فعال ضد تلف الكبد المستحدث بواسطة الأسيتوأمينوفين.

Introduction

Acetaminophen (APAP) is a most commonly used analgesic and considered to be safe at therapeutic doses. It effectively reduces fever and mild-to moderate pain. However, APAP overdose causes severe hepatotoxicity that leads to liver failure in both humans and experimental animals (Boyd and Bereczky, 1966; Boyer and Rouff, 1971; Kaplowitz, 2005). Although a large dose of APAP is directly conjugated with glucuronic acid or sulfate and excreted, a significant amount of APAP is metabolized by the

cytochrome P450 system and this leads to the formation of a reactive metabolite, presumably *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which reacts rapidly with glutathione (GSH) (Nelson, 1990). Active oxygen molecules such as superoxide and hydroxyl radicals have been demonstrated to play important role in the inflammation process produced by ethanol, carbon tetrachloride or paracetamol (Halliwell, 1994). Since oxidative stress and GSH depletion contributed to APAP -induced liver injury; the agent(s) with antioxidant property and/or GSH reserving ability may provide preventive effect against the progression of lipid peroxidation and hepatocellular injury (Hsu *et al.*, 2008). The liver plays an essential role in the metabolism of foreign compounds entering the body. Human beings are exposed to these compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances in the occupational environment. In addition, human beings consume a lot of synthetic drugs during diseased conditions which are alien to body organs. All these compounds produce a variety of toxic manifestations (Athar *et al.*, 1997). Liver is the most important organ concerned with the biochemical activities in the human body (Ramaiah, 2007). It has great capacity to detoxicate toxic substances and synthesize useful principles (Kitteringham, 1998). Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. A large number of xenobiotics are reported to be potentially hepatotoxic. Some examples are APAP, tetracycline, ethanol and carbon tetrachloride (Yen and Wu, 2000). Hepatotoxins may react with the basic cellular constituents like proteins, lipids, RNA and DNA and induce almost all types of lesions of the liver (Sallie *et al.*, 2009).

Honey can be considered as a dietary supplement as it contains some important components including α -tocopherol, ascorbic acid, vitamins, organic acid, flavonoids, phenolics enzymes and other phytochemical compounds (Mendes *et al.*, 1998). The use of honey in the treatment and prevention of numerous diseases has been documented (Castaldo and Capasso, 2002). Many authors demonstrated that honey serves as a source of natural medicine, which is effective in reducing the risk of heart disease, cancer, immune system decline, cataracts, different inflammatory processes etc. (Bertoncelj *et al.*, 2007). However, since some of these diseases are a consequence of oxidative damage, it seems that part of the therapeutical properties of honey products is due to their antioxidant capacity (Hegazi and Abd El-Hady, 2009). Furthermore, honey as a source of antioxidants, has been proven to be effective against deteriorative oxidation reaction in food (McKibben and Engeseth, 2002). Pure honey has bactericidal activity against many enteropathogenic organisms (Jeddar *et al.*, 1985) and the administration of a honey three times a day was found to correct anaemia in more than 50% of the patients (Salem, 1981). Many studies have shown that honey reduces the secretion of gastric acid (Kandil *et al.*, 1987) as well as it is an effective treatment of wounds (Bulman, 1953). Honey has been shown to be an effective treatment for conjunctivitis in rats (Al-Waili, 2004). Honey protects liver against oxidative damage induced by APAP in rat (Ayyavu *et al.*, 2009).

The aim of the present study is to evaluate the possible protective effect of honey on APAP induced liver damage in mice.

Material and methods

Materials

APAP supplied by Ibn hayan company/Syria. Honey sample was obtained from Dohuk city. Working reagents for ALT and AST are 2-oxoglutarate, L-Alanine, lactic

dehydrogenase (LDH), β -nicotinamide adenine dinucleotide hydrogen (NADH), Tris buffer solution, ethylene diamine tetra acetic acid disodium salt (EDTA), and L-Aspartate.

Experiment protocol:

18 males mice weighing (30 ± 2 gm) were used throughout the experimental study and taken from animal house in Pharmacy Collage/Baghdad University. The experimental animals were maintained under controlled environmental conditions. They were provided a free access to standard pellet diet and tap water. The animals were divided randomly into three groups each group consisting of six animals. Group (I) mice served as control. Group (II) mice (intoxicated group) were received single dose of APAP (500mg kg^{-1} body weight, i.p.) (Cynhia, 2005). Group (III) mice were pre-treated with honey (600mg kg^{-1} body weight, p.o.) for 8 successive days, followed by intoxication with APAP (500mg kg^{-1} body weight, i.p.). Blood samples were collected by cardiac puncture from ether anesthetized mice and used to determine the serum ALT and AST. Then the animals were sacrificed and a section of liver was dissected out and fixed in 10 % formalin solution for histopathological study. Liver sections were prepared and stained with eosin and hematoxyllin and examined under ordinary light microscope.

Statistical analysis

Data analyzed using one-tailed t-test. The difference showing a level of $P < 0.05$ was considered to be statistically significant.

Results

Effect of honey on serum ALT and AST in APAP induced hepatotoxicity in mice

Serum ALT and AST levels are susceptible to hepatotoxin and serve as markers of liver damage which promotes the release of such serum enzymes from hepatocytes into blood stream. The effects of pretreatment with honey on the APAP-induced elevation of serum enzymes ALT and AST was shown in Table 1. APAP increased ALT (91 ± 6.7 IU L $^{-1}$) and AST (72 ± 9 IU L $^{-1}$) level significantly ($P < 0.05$) compared with control group which indicates that APAP causes damage to hepatocytes. Pretreatment with honey prior to APAP administration decreased the increment of ALT level (81.66 ± 13.5 IU L $^{-1}$) and AST (63 ± 21 IU L $^{-1}$) compared with intoxicated group; however, this decrease was not significant.

Effect of honey on histopathological changes in APAP hepatotoxicity in mice

Histologically, control animals (Group I) showed normal hepatic appearance. Group II (APAP treated mice) revealed extensive damage characterized by degeneration and necrosis in the hepatocytes with vacuolation of the cytoplasm. However, the section of liver in mice pre-treated with honey (Group II) showed focal area of degeneration dispersant in-between hepatocytes without necrosis (Figure 1).

Table 1. Effect of honey on serum enzymes level in APAP induced hepatotoxicity in mice.

Group	ALT IU/L	AST IU/L
Control	35.3 ± 10.3	17.6 ± 1.74
APAP treated	91 ± 6.7*	72 ± 9*
APAP + Honey	81.66 ± 13.5	63 ± 11

Each value represents the mean ± SD of six animals. *significant difference at $P < 0.05$ compared with the control. #significant difference at $P < 0.05$ compared with the APAP treated group.

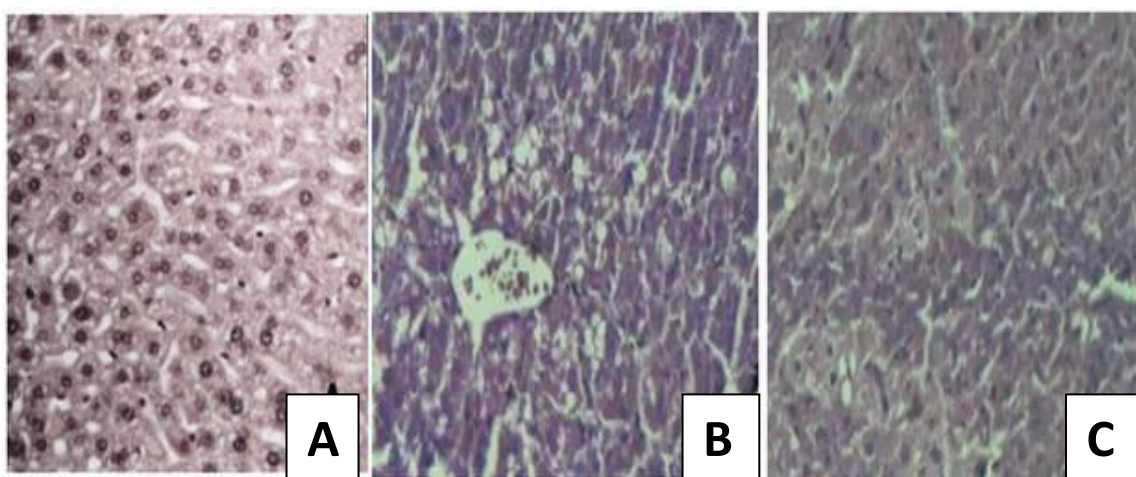


Figure 1. A – Normal histological appearance of the liver of control mice, B – APAP induced hepatotoxicity in mice, C – APAP + honey pretreatment.

Discussion

Various pharmacological or chemical substances are known to cause hepatic injuries such as APAP, CCl₄ and dimethylnitrosamine; excessive dose exposure to these hepatotoxins may induce acute liver injury characterized by abnormality of hepatic function and degeneration, necrosis or apoptosis of hepatocyte (Higuchi and Gores, 2003). With respect to APAP dependent hepatotoxicity, it is generally accepted that P450-dependent bioactivation of APAP is a main cause of potentially fulminant hepatic necrosis upon administration or intake of lethal dose of APAP (Bailey *et al.*, 2003; Lee, 2004). APAP hepatotoxicity is the result of a cascade of interrelated biochemical events (Bartlett, 2004). The hepatotoxic effect of APAP observed in group (II) mice which may be due to increased free radical production caused by administration of APAP. It is well known that a large dose of APAP causes hepatic GSH depletion because NAPQI reacts rapidly with GSH, which consequently exacerbates oxidative stress in conjunction with

mitochondrial dysfunction and glutathione peroxidase (GPx) that present in the cells can catalyze this reaction (Oz *et al.*, 2004). ALT is present in very high amounts in liver and kidneys, and in smaller amounts in skeletal muscles and heart. Although serum levels of both AST and ALT become elevated whenever a disease processes affecting liver cells integrity, ALT is the more liver-specific enzyme (Tietz, 1999). AST is distributed in all body tissues, but greatest activity occurs in liver, heart, skeletal muscle and in erythrocytes (Tietz, 1995). In the present study, honey exhibited hepatoprotective effect as evident by a decrease in ALT and AST and histopathological examination of liver section. Although the decrease in ALT and AST was not significant, Group II mice exhibited significant liver protection against APAP-induced liver damage as evident by the absence of necrosis in the hepatocyte (Figure 1). This result is consistent with previous study in which honey was observed to have a potent hepatoprotective action upon APAP-induced oxidative stress and liver toxicity in rat as demonstrated by a significant decrease in serum ALP and AST and total bilirubin level in rat with APAP hepatotoxicity (Ayyavu *et al.*, 2009). It seems that part of the therapeutical properties of honeybee products is due to their antioxidant capacity (Hegazi and Abd El-Hady, 2009). Honey contains some important components including α -tocopherol, ascorbic acid, vitamins, flavonoids, and other phytochemical compounds which considered as antioxidant compound (Mendes *et al.*, 1998). Vitamin C (ascorbic acid) is considered the most important water-soluble antioxidant in extracellular fluids, as it is capable of neutralizing reactive oxygen species (ROS) in the aqueous phase before lipid peroxidation is initiated. Vitamin E is a major lipid-soluble antioxidant, and is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation (Halliwell, 1994; Jacob, 1995). Furthermore, vitamin C raises intracellular glutathione levels thus playing an important role in protein thiol group protection against oxidation (Naziroglu and Butterworth, 2005). There is an evidence to suggest that α -tocopherol and ascorbic acid function together in a cyclic-type of process (Kojo, 2004). Vitamin C (ascorbic acid) cooperates with vitamin E to regenerate α -tocopherol from α -tocopherol radicals in membranes and lipoproteins (Carr and Frei, 1999). The flavonoids are important factors in human healthy by decreasing the risk of disease and increase the activity of vitamin C, inhibition platelet aggregation, and its action as anti inflammatory, anticancer, antioxidant substance (Cook and Samman, 1996; Craige, 1999). The most reported activity of flavonoids is their protection against oxidative stress (Rice-Evans, 2001).

In conclusion, honey may reduce the hepatotoxic effect of APAP and it can be used as an effective hepatoprotector against APAP induced liver damage. However, longer honey treatment (10-14 days) and large-scale study is awaited and more parameters can be measured to confirm the hepatoprotective effects of honey as it was demonstrated in this experimental study.

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