Protective role of *Nigella sativa* oil on renal damage induced by acetaminophen in male rats

Zena, M. Hamad
College of Basic Education, Wassit University, Iraq.
E-mail: Zena_alshammary@yahoo.com
Received: 16/9/2012; Accepted: 19/2/2013

Summary

Acetaminophen also called paracetamol is commonly used as analgesic and antipyretic agent which in high doses causes liver and kidney damage in man and animals. *Nigella sativa* oil have antioxidant properties. Thirty adult male rats were used and randomly divided into three equal groups. Group (A) untreated and served as control group; Group (B) rats were orally intubated (by gavages needle) acetaminophen suspension (150mg/kg B.W). Group (C) rats were given orally acetaminophen suspension (150mg/kg) plus 1ml/kg B.W of *Nigella sativa* oil for 42 days in both treated group. Fasting blood samples were collected at 21 and 42 days of experiment to study the following parameters: Serum creatinine concentration and blood urea nitrogen concentration. The results revealed a significant increase of acetaminophen group in serum creatinine and blood urea nitrogen concentrations as compression with GA. Animals treated with *Nigella sativa* oil plus acetaminophen (C) showed a significant decline in serum creatinine and blood urea nitrogen concentrations. In conclusion, the acetaminophen was effective in induction of oxidative stress and change in some biological markers related to kidney disease. Also it seems that *Nigella sativa* oil exerts protective actions against the damaging effect of acetaminophen

Keywords: ***Nigella sativa* oil, Kidney damage, Acetaminophen, Rats.**

Introduction

Acetaminophen (Paracetamol) *N*-acetyl-*p*-aminophenol; APAP is also known as paracetamol, is widely used as prescription and over the counter analgesic and antipyretic agent that is safely employed for a wide range of treatments (1). Overdose of APAP is often associated with hepatic and renal damage in both humans and experimental animals (2). Kidney is the second target organ of acetaminophen toxicity and renal dysfunction occurs among patients with marked hepatic injury; however, acetaminophen nephro -toxicity after acute overdose might occur in the absence of hepatotoxicity (3). The initial step of its toxicity is formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) by cytochorom P450 which at therapeutic doses is removed by conjugation with gluthation reduced (GSH) (4). High doses of acetaminophen result in the depletion of cellular GSH which allows NAPQI to bind to cellular proteins and initiate lipid peroxidation leading to renal injury (5). Acetaminophen-induced renal injury could be due to hepatic-derived acetaminophen metabolites, particularly GSH conjugates (6).

Studies are going on throughout the world for the search of protective molecules that would provide maximum protection to the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body (7). A number of herbs are traditionally used in different countries during drug or toxin induced hepatic and renal disorders (8). *N. sativa* occupies a unique position among the herbal products of Southeast Asia as a natural remedy for a number of illnesses. Its anti-bacterial, hypolipidaemic, antidiabetic and anti-hypertensive properties have been reported (9 and 10). Its Arabic name is Habat-ul-Sauda and its English name is Black cumin (11). The seeds or compounds isolated have been found to be useful in a number of models of nephrotoxicity. The phytochemical, pharmacological and toxicological properties of *N. sativa* have recently been reviewed (12). Attempts were made to obtain agents that could ameliorate or potentiate the nephrotoxicity of acetaminophen (13). Among these agents, extract of medicinal plants like garlic oil (14), and *curcuma longa* (15) have been reported to possess properties to ameliorate acetaminophen induced nephro -toxicity. One common feature of the herbal agents is that they all have antioxidant properties (16). A potential therapeutic

approach to ameliorate acetaminophen-induced renal damage would have very important clinical consequence (17). The present study designed to investigate the effect of *Nigella Sativa* oil on kidney damage induced by acetaminophen in male rats.

Materials and Methods

Thirty adult male albino Wister rats with a body weight 180-200 gm and aged ranged between (2.5-3) months were used. The animals were handled under standard laboratory conditions of a 12-hour light /dark cycle. Food and water available *ad libitum* along the experimental period. The animals were randomly divided into three equal groups. GA served as control group, GB rats were orally intubated (by gavages needle) acetaminophen suspension (paracetamol S.D.I Iraq) at a dose 150mg/kg B.W at concentration 500mg (18). GC rats were given orally acetaminophen suspension 150mg/kg at concentration 500mg plus 1 ml /kg B.W of *Nigella sativa* oil (kut manufactures information) for 42 day in both treated group (19). Fasting blood samples were collected at 21 and 42 days of experiment. Blood were drawn via cardiac puncture technique from anesthetized rats (intramuscular injection of ketamine 90 mg/kg B.W and xylazine 40 mg/kg B.W) and the serum was used for the assay of serum creatinin (SC) and blood urea nitrogen (BUN) concentration**.** Data were performed on the basis of analysis of variance (ANOVA) using significant level of (P<0.05). Specific group differences were determined using least significant differences (LSD), (20).

Results and Discussion

Serum blood urea nitrogen significantly increased (P<0.05) in Acetaminophen treated GB at show day 21 and 42 of the experiment comparing to *Nigella sativa* oil treated GC and GA. There was a significant reduction (P<0.05) in BUN concentration after 21 days of treatment in group compared with the GB and GA. At the end of the experiment day 42 a significant reduction (P<0.05) in BUN was observed after orally administration of *Nigella sativa* oil concurrently with acetaminophen in GC comparing to GB (Table, 1).

Table, 1: Blood Urea Nitrogen mg/dl concentration in male rats orally in experimental group

Groups Time (Days)	GA Control BUN	GB Acetaminophen 150mg/kg B.W BUN	GC Acetaminophen 150mg/kg B.W + *Nigella sativa* oil (1 ml / kg B.W) BUN
21	23.22 ±1.17 c	43.67 ±5.51 a	39.27 ±2.78 b
42	22.5 ±1.03 c	48.55 ±5.81 a	32.57 ±2.56 b

Small letters denote differences between group at the level (P<0.05).

The result of (Table, 2) show a significant elevation (P<0.05) in SC of experimental group at the 21 and 42 days of experiment. However, at the end of the experiment, *Nigella sativa* oil caused significant decrease (P<0.05) in mean value of SC concentration in GC comparing to other groups.

Table, 2: Serum creatinine mg/dl concentration in male rats orally administered by acetaminophen and *Nigella sativa* oil compared with control group.

Groups Time (Days)	GA Control SC	GB Acetaminophen 150mg/kg B.W SC	GC Acetaminophen 150mg/kg B.W+ *Nigella sativa* oil (1 ml /kg B.W) SC
21	0.53 ±0.60 c	1.29 ±1.47 a	0.77 ±0.86 b
42	0.51 ±0.55 c	1.39 ±1.51 a	0.66 ±0.72 b

Small letters denote differences between group at the level (P<0.05).

The results of the present study showed that daily oral intubation of acetaminophen over dose for 42 days caused a significant elevation in SC and serum BUN concentration (Tables, 1 and 2). The elevation of BUN and creatinine are considered for investigating drug induced nephrotoxicity in animals and man (21). The reason behind acetaminophen toxicology is the CYP-mediated conversion of acetaminophen to a highly reactive quinone imine, N-acetyl-p-benzoquinone imine. The fundamental role of NAPQI in the toxicity of acetaminophen has been supported by (22).

Blood urea nitrogen is found in the liver protein that is derived from diet or tissue

sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance (23). Elevation of urea and creatinine levels in the serum was taken as the index of nephrotoxicity (24). Urea level could be increased by many other factors such as dehydration, anti diuretic drugs and diet, whilst creatinine is, therefore, more specific to the kidney, since kidney damage is the only significant factor that increases serum creatinine level (25). Creatinine is derived from endogenous sources by tissue creatinine breakdown (23). In the present study, administration of nephrotoxic doses APAP to rats resulted in development of oxidative stress damage in renal tissues, also APAP induced nephrotoxicity showed a significant ($P<0.05$) increase in the serum urea and creatinine concentrations in the GB rat when compared to the normal GA. Therefore significant increases in urea and creatinine levels reported in this study (Table, 2) the kidney was adversely affected by acetaminophen administration. Kidney dys -function and nephrotoxicity induced by acetaminophen in present investigation are consequences of oxidative stress.

Nigella sativa oil exerts protective actions against damaging effect acetaminophen on renal system causing significant decrease in kidney biomarkers (SC and BUN). Such increase in kidney function biomarkers after oral administration *Nigella sativa* is correlated with (26). Pretreatment of acetaminophen-intoxicated rats with *Nigella sativa* oil normalized the levels of urea and creatinine. *Nigella sativa* is composed of about 100 pharmacologic active ingredients, one of the most important of which is thymoquinone TQ, the main constituent of *Nigella sativa* oil, ameliorated the severity of ifosfamide-induced renal damage (27). Thymoquinone the main compound of the essential oil inhibit non enzymatic lipid peroxidation in liposomes (28). It was shown that thymoquinone has antioxidant effect. Oxidative stress could exaggerate kidney toxicity induced by acetaminophen. The other ingredients of *Nigella sativa* can exert beneficial effects on the renal toxicity induced by acetaminophen (29). *Nigella sativa* acts in the kidney as a potent scavenger of free radicals to prevent or inhibit the toxic effects of acetaminophen on kidney function. Administration of *Nigella sativa* oil was effective in ameliorating the biochemical and physiological indexes of nephrotoxicity during the administration of the nephrotoxic drug acetaminophen.

In conclusion, it is plausible to suggest that Acetaminophen-induced a case of renal dysfunction, through an increase in serum creatinine and blood urea nitrogen concentra -tion, but administration of *Nigella sativa* oil at this dose exerted renal protective action against acetaminophen induce renal dysfunction.

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الدور الوقائي لزيت الحبة السوداء على التلف الكلوي المستحدث بالاسيتامينوفن في ذكور الجرذان

زينة محمد حمد
كلية التربية الاساسية، جامعة واسط، العراق.
E-mail: Zena_alshammary@yahoo.com

الخلاصة

الاسيتامينوفين الذي يدعى الباراسيتامول أيضاً، وعادة ما يستعمل كمسكن للآلام وخافض للحرارة لكن الجرعات العالية تسبب تلف الكبد والكلى في الانسان والحيوانات. زيت الحبة السوداء له خواص مضادة للأكسدة. استعمل 30 من ذكور الجرذان وقسمت عشوائياً على ثلاثة مجاميع متساوية:اعطيت المجموعة الاولى الماء العادي وعدت كمجموعة سيطرة ،اما المجموعة الثانية فقد جرعت محلول الاسيتامينوفين وبجرعة مقدارها 150 ملغم/كغم من وزن الجسم أما المجموعة الثالثة فقد أعطيت فضلاً عن الاسيتامينوفين زيت الحبة السوداء وبجرعة 1 مل /كغم من وزن الجسم لمدة 42 يوما. جُمعت عينات الدم في الأيام 21 و 42 من التجربة لدراسة المؤشرات الآتية: تركيزالكرياتنين ونتروجين يوريا الدم. أظهرت النتائج حدوث زيادة معنوية في تركيز الكرياتنين ونتروجين يوريا الدم لمجموعة الاسيتامينوفين بالمقارنة مع مجموعة السيطرة. من جهة أخرى أظهرت المجموعة المعاملة بزيت الحبة السوداء فضلاً عن الاسيتامينوفين وجود انخفاض معنوي في تراكيز الكرياتنين ونتروجين يوريا الدم، نستنتج من الدراسة أن الاسيتامينوفين احدث إجهاد تأكسدي وتغير في بعض المعايير الحيوية المتعلقة بأمراض الكلية. ويمارس زيت الحبة السوداء التأثير الوقائي ضد أضرار الاسيتامينوفين.

الكلمات المفتاحية: زيت الحبة السوداء، التلف الكلوي، الاسيتامينوفين، الجرذان.