Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Effects of Caffeine on Renal Toxicity Induced by Diethylnitrosamine

Marzie Pashmforoosh,[1] Annahita Rezaie,[2] Mojtaba Haghi-Karamallah,*[3] Ali Fazlara,[4] Ali Shahriari,[5] Hosein Najafzadeh[5]

1. Resident of Medical Pharmacology, Faculty of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Ph.D Student of Biochemistry, International Campus, Shahid Soudoghi University of Medical Sciences, Yazd, Iran
4. Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
5. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Article information

Article history:
Received: 2 Aug 2013
Accepted: 2 Oct 2013
Available online: 10 Dec 2013
ZJRMS 2015 Jan; 17(1): 7-9

Keywords:
Diethylnitrosamine
Caffeine
Toxicity
Rat
Kidney

*Corresponding author at:
Veterinarian, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
E-mail:
mojtabahaghi@ymail.com

Abstract

***Background*:** The kidney by the way is an essential organ and part of the urinary system and serves as natural filter of blood and removal of wastes, among other functions. The objective of this research is to investigate the effect of caffeine on renal toxicity induced by diethylnitrosamine (DEN), in rats.

***Materials and Methods*:** In this experimental study, 40 female rats were divided into 4 groups with 10 animals in each group. Group I (control group), group II were injected with a single dose of DEN (200 mg/kg, i.p.), group III were injected i.p. caffeine (100 mg/kg), daily for 4 weeks, group IV received the same treatment as group 2 of DEN and received daily caffeine as group 3. After 30 days, blood was collected for analyzing level of creatinine and blood urea nitrogen (BUN).

***Results*:** The mean serum BUN and creatinine levels were significantly higher in DEN treated control group in comparison to those of base line control. Again, these levels were significantly lower in caffeine pretreated and DEN treated group (groups II, III, IV) when compared to those of DEN treated group (group I).

***Conclusion*:** Caffeine may have some nephroprotective effect against DEN induced nephrotoxicity.

Introduction

N-nitroso compounds (NOCs) are one of the important groups of carcinogens frequently present in human environment and food chain. Nitrosamines are formed endogenously from nitrate and nitrite under certain conditions such as the strong acidic pH of the stomach [1, 2]. Both environmental and food born N-nitrosamines pose a health risk for human and animals. Diethylnitrosamine is experimentally used to induce liver carcinoma and study the mechanisms of cytotoxic injury [3]. Diethylnitrosamine is reported to undergo metabolic activation by cytochrome P450 enzymes to form reactive electrophones which cause oxidative stress leading to cytotoxicity, mutagencity and carcinogenicity [1]. DEN is reported to induce generation of free radicals leading to oxidative stress and cell injury through its metabolized end product [4]. Various plants have been tested and found to be effective against diethyl nitrosamine induced carcinogenesis and toxicity [5].

Caffeine (1, 3, 7-trimethylxanthine) is a purine alkaloid present in many popular beverages, including cocoa, tea and coffee [6]. Caffeine and other methylxanthines are used in clinical medicine as diuretics, analgesics and muscle relaxants; and they can aid in the treatment of brain disorders such as headaches and Parkinson's disease [7, 8]. The effect of caffeine on biological system has been examined and the results differ according to the dose tissue and duration of treatment. Some of the effects of caffeine may favor the production of free radicals and lead to a subsequent increase of lipid peroxidation by increasing oxidative stress [9, 10]. More recent observations suggest that it can also act as an antioxidant. The suggestions are largely based on chemical studies showing it to be able to scavenge reactive oxygen species (ROS), particularly the hydroxyl radical (OH), known to be generated in the body by irradiation with various electromagnetic frequencies such as exposure to UV, as well as by many ambient physiologic reactions involving oxygen utilization [11, 12].

In light of these observations, it was decided to evaluate the efficacy of caffeine, as an antioxidant against diethylnitrosamine induced renal damage.

Materials and Methods

Preparation of Chemical: Diethylnitrosamine (Sigma Aldrich, USA), and caffeine were obtained from sigma, USA.

Animals and treatments: For this experimental study, 40 female albino Wistar rats weighting 180±5 g were kept in the laboratory under constant conditions of temperature (24±2ºC) for at least 1 week before and through the experimental work, being maintained on a standard diet and water were available ad libitum. The animals were maintained in accordance with the guidelines prescribed by the faculty of sciences and the study was approved by

the Animal Ethics Committee of the University of Shahid Charmin University, Iran. The experimental rats were divided into 4 groups:

Group I (control): Animals were fed on the standard diet and were served as control group. Group II (DEN): Rats were injected intraperitonealy with single dose of DEN 200 mg /kg [13, 14]. Group III (Caff): Animals were i.p. given 100 mg/kg caffeine, daily for 4 weeks. Group IV (DEN+Caff): Rats were injected with DEN followed by i.p. caffeine, daily for 4 weeks.

Biochemical assays: For biochemical study sera were obtained by centrifugation of the blood samples and stored at 20ºC until assayed for the biochemical parameters. The level of creatinine and BUN was assayed in serum were determined using commercially available kits (Pars azmoon Tehran, Iran).

Statistical analysis: Results are expressed as the mean ±standard error of mean (SEM). Within group comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by student's *t*-test using SPSS-11. A probability level of less than 5 ($p<0.05$) was considered significant.

Results

Table 1 and table 2 show effect of single, daily 100 mg/kg bodyweight of i.p. caffeine on the average weight in DEN (200 mg/kg) nephrotoxic rats treated for 30 days. As indicated in the table, repeated i.p. injection with DEN induced significant progressive weight loss in the treated rats. However, weight loss was significantly enhanced by caffeine treatment in dose related fashion and the other hand, kidney weight also changes significantly by DEN treatment while recovery was seen by caffeine.

Figure 1 shows the status of serum creatinine and BUN in control and experimental animals. Diethylnitrosamine (group II) induced renal toxic is shown by a 2 fold increase in the level of creatinine and BUN in the serum of rats as compared to controls (group I). This increased level of creatinine and BUN in serum due to diethylnitrosamine challenge was significantly decreased on post treatment with caffeine (group IV) for 4 weeks.

Table 1. Average body weight changes in the caffeine group

Average body weight (g)	Day 0 (mean±SEM)	Day 30 (mean±SEM)
I	139±10.19	149.83±8.23
II	140.33±7.58	128.67±7.78*
III	141.83±10.26	148.56+ 9.22
IV	140.33±7.53	149.67± 7.63

*$p<0.05$

Table 2. Average kidney weight changes in the caffeine group

Group	Kidney wet weight (g) (Mean±SEM)
I	1.6 ±0.05
II	1.2±0.03*
III	1.7±0.13
IV	1.3±0.55

*$p<0.05$

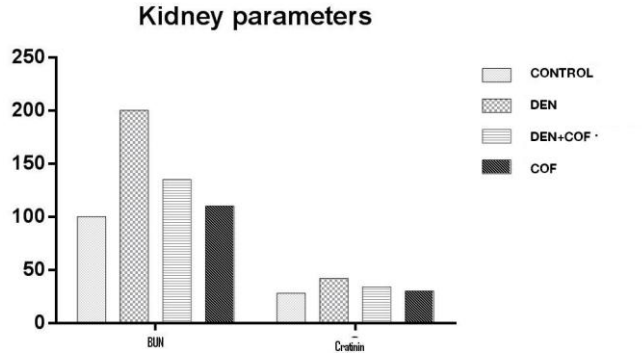

Figure 1. Effect of caffeine on creatinine mg/dL and BUN levels in serum during diethylnitrosamine induced renal toxicity
Results are given as Mean±SEM, for 10 rats. Comparisons are made between: control rats (Group I); DEN treated rats (Group II); $p<0.05$.

Discussion

In the present study, serum levels of BUN and creatinine were significantly higher in diethylnitrosamine treated control and in experimental group in comparison to those of baseline control group. Again, significantly lower levels of these parameters were observed in experimental group when compared to those of N-diethylnitrosamine treated control group.

The kidney is an essential excretory organ of our body, plays a dominant role in homeostasis by excreting the metabolic waste products and excess necessary substances. Metabolites of the drugs that are excreted from kidney may also cause cellular damage leading to kidney dysfunction. Several xenobiotic substances exert their toxic effects by one or more common pathogenic mechanism that can produce nephrotoxicity [15]. Kidney disease is one of the commonest causes of hospitalization in most of the countries. Increase in the levels of blood urea and creatinine is the principal diagnostic criteria of renal failure. Severe and progressive uremia may result in death [16].

Diethylnitrosamine, one of the most important environmental carcinogen, has been suggested to cause the generation of ROS resulting in oxidative stress and cellular injury [17]. Diethylnitrosamine metabolized by cytochrome p450 generates a highly reactive free radical, and initiates lipid peroxidation of the cell membrane of the endoplasmic reticulum and causes a chain reaction. These reactive oxygen species can cause oxidative damage in DNA, proteins and lipids [1, 18].

The present study documents the antioxidant of the caffeine against kidney injury induced by DEN in rats. In this study, diethylnitrosamine administration to rats lead to a marked elevation in the levels of serum BUN and creatinine which is indicative of kidney damage, as previously reported [13, 19]. A single intraperitoneal dose of N-diethylnitrosamine (200 mg/kg body weight) increases microtonal lipid peroxidation and the activity of xanthenes oxidize and decreases the activities of renal antioxidant enzymes via, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase, phase II metabolizing enzymes such as glutathione-S-transferase and quinone reductase and causes depletion in the level of renal glutathione content.

A sharp increase in BUN and serum creatinine has also been observed [19].

Treatment with caffeine significantly reduced the level of the above marker parameters in diethylnitrosamine treated rats. This indicates that caffeine tends to prevent kidney damage. Haze et al. reported that administration of rats with caffeine alleviated the deleterious effect of cisplatin on kidney. They added that caffeine as an agent for reducing a renal toxicity with three mechanism, antioxidant, diuretic activities and blocking organic action transporter [20].

Caffeine is rich in photochemical derivatives such as triterpenes, flavonoids or polyphones. Many studies reported that the preventive effects of caffeine are attributed to its antioxidant activity [21]. Huber et al. reported that kahweol and cafestol phenol diterpenes of caffeine inhibit lipid peroxidation [22]. Lee et al. reported protective effects of caffeine on hepatotoxicity induced by carbon tetrachloride (CCl4) [23]. In conclusion, the present results showed that caffeine alleviates the renal toxicity induced by DEN in albino rats. This effect of caffeine may be attributed to the anti oxidant activity of one or more of its constituents.

Acknowledgements

This article was written based on the thesis of Dr Mojtaba Haghi-Karamallah with No. 9158821 and was supported by Shahid Chamran University.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Shahid Chamran University of Ahvaz.

References

1. Archer MC. Mechanisms of action of N-nitroso compounds. Cancer Surv. 1989;8(2):241–50.
2. Jakszyn P, Gonzalez CA. Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. World J Gastroenterol. 2006;12(27):4296–303.
3. . Skog K. Problems associated with the determination of heterocyclic amines in cooked foods and human exposure. Food Chem Toxicol. 2002;40(8):1197–203.
4. Bansal AK, Trivedi R, Soni GL, Bhatnagar D. Hepatic and renal oxidative stress in acute toxicity of N-nitrosodiethylamine in rats. Indian J Exp Biol. 2000;38(9):916–20.
5. Ahmed S, Rahman A, Mathur M, Athar M, Sultana S. Anti-tumor promoting activity of Asteracantha longifolia against experimental hepatocarcinogenesis in rats. Food Chem Toxicol. 2001;39(1):19–28.
6. Ritchie JM. The xanthenes. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman's the pharmacological basis of therapeutics. 12th ed. New York: McGraw Hill; 2010. pp. 367–78.
7. Kolayli S, Oak M, Kuku M, Riza A. Does caffeine bind to metal ions. Food Chem. 2004;84(3):383–8.
8. Chen X, Ghribi O, Geiger JD. Caffeine protects against disruptions of the blood-brain barrier in animal models of Alzheimer's and Parkinson's diseases. J Alzheimers Dis. 2010;20 Suppl 1:S127–41.
9. Jewett SL, Eddy LJ, Hochstein P. Is the autoxidation of catecholamines involved in ischemia-reperfusion injury? Free Radic Biol Med. 1989;6(2):185–8.
10. Dianzani MU, Muzio G, Biocca ME, Canuto RA. Lipid peroxidation in fatty liver induced by caffeine in rats. Int J Tissue React. 1991;13(2):79–85.
11. Shi X, Dalal NS, Jain AC. Antioxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. Food Chem Toxicol. 1991;29(1):1–6.
12. Devasagayam TP, Kamat JP, Mohan H, Kesavan PC. Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. Biochim Biophys Acta. 1996;1282(1):63–70.
13. Atakisi O, Atakisi E, Ozcan A, Karapehlivan M, Kart A. Protective effect of omega-3 fatty acids on diethylnitrosamine toxicity in rats. Eur Rev Med Pharmacol Sci. 2013;17(4):467–71.
14. Shaarawy SM, Tohamy AA, Elgendy SM, Elmageed ZY, Bahnasy A, Mohamed MS, et al. Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. Int J Biol Sci. 2009;5(6):549–57.
15. Naughton CA. Drug-induced nephrotoxicity. Am Fam Physician. 2008;78(6):743–50.
16. Anderson TM, Jones DB. Kidneys. In: Scotti WA, Anderson TM, editors. Synopsis of pathology. 9th ed. USA: Mosby Company; 1976. pp. 772–6.
17. Bartsch H, Hietanen E, Malaveille C. Carcinogenic nitrosamines: free radical aspects of their action. Free Radic Biol Med. 1989;7(6):637–44.
18. Vitaglione P, Morisco F, Caporaso N, Fogliano V. Dietary antioxidant compounds and liver health. Crit Rev Food Sci Nutr. 2004;44(7-8):575–86.
19. Khan N, Sharma S, Alam A, Saleem M, Sultana S. Tephrosia purpurea ameliorates N-diethylnitrosamine and potassium bromate-mediated renal oxidative stress and toxicity in Wistar rats. Pharmacol Toxicol. 2001;88(6):294–9.
20. Khazaei M, Bayat PD, Ghanbari A, Khazaei S, Feizian M, Khodaei A, et al. Protective effects of subchronic caffeine administration on cisplatin induced urogenital toxicity in male mice. Indian J Exp Biol. 2012;50(9):638–44.
21. Cavin C, Mace K, Offord EA, Schilter B. Protective effects of coffee diterpenes against aflatoxin B1-induced genotoxicity: mechanisms in rat and human cells. Food Chem Toxicol. 2001;39(6):549–56.
22. Huber WW, Scharf G, Rossmanith W, Prustomersky S, Grasl-Kraupp B, Peter B, et al. The coffee components kahweol and cafestol induce gamma-glutamylcysteine synthetase, the rate limiting enzyme of chemoprotective glutathione synthesis, in several organs of the rat. Arch Toxicol. 2002;75(11-12):685–94.
23. Lee KJ, Choi JH, Jeong HG. Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice. Food Chem Toxicol. 2007;45(11):2118–25.