International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 100-105 www.ijprajournal.com ISSN: 2249-7781

The Protective Effects of Betacarotene from Carrot (Daucuscarota L.)on Paracetamol Induced nephrotoxicity in Male Laboratory Rats

[1]Muhamad Aldino Putra, [2*]SonlimarMangunsong, [3]Muhamad Taswin, [4]Sarmalina Simamoraand[5]Pododjoyo
[1,2,3,4,5]*Health of Polytechnic Palembang*

Submitted: 01-01-2022 Accepted: 10-01-2022

ABSTRACT
Paracetamol overdose can cause nephrotoxicity with high of uric acid level as one of the possible mechanisms mediating the event. This study aimed to examine the effect of short-term exposure to therapeutic doses of paracetamol and over-exposure, in male rat and to investigate whetherbetacarotene was effective at preventing paracetamol nephrotoxicity induced by these exposures
MethodsScreening of the betacarotene was done. Twenty four adult male rats were divided into six groups (n= 4 in each group). Group A (control negatif) animals received feed normal for ten days, group B (paracetamol group) received allopurinol 1,8 mg/200gBB, and paracetamol single dose/day for ten days 300 mg/200gBB orally. Group C received paracetamol 300 mg/200kgBB, Group D received betacarotene 2 mg/200gBB and paracetamol 300 mg/200kgBB, Group E received betacarotene 4 mg/200gBB and paracetamol 300 mg/200kgBB, and group Freceived betacarotene 8 mg/200gBB and paracetamol 300 mg/200kgBB. respectively, for ten consecutive daysdays of administration. Blood samples withdrawn from the tail for uric acid level examination using auotocheck tools on day 0, 7th and 10th.
Result Screening of betacarotene showed presence of nephroprotectivepytochemicals. Paracetamol administration resulted in significant elevation of uric acid markers. Treatment with betacarotene at doses of 2 and 4 mg/200gBB prevented the Paracetamol-induced nephrotoxicity and impairments of the kidney, as evidenced by a significantly reduced ($P<0.05$) level of uric acid.Betacarotene ameliorated the effect of paracetamol by reducing the markers as well as reversing the paracetamol-induced changes.
Conclusion Carrot contains betacarotene,nephroprotective phytochemicals and may be useful in preventing kidney damage induced by paracetamol.
Keywords: paracetamol, uric acid, male rat, betacarotene, nephroprotective

I. INTRODUCTION

Daucus carota, commonly known as carrot, is a popular medicinal plant with various pharmacological activities in traditional and modern phytotherapy including antioxidant, analgesic, anti-inflammatory, antimicrobial, antifungal, diuretic, lithontripic, emmenagogue, intra occular hypotensive, gastroprotective, hepatoprotective, aphrodistic, nephroprotective, antispasmodic, anticancer, antiestrogenic, cardioprotective, and wound healing activities (Bahramiet al, 2018). No serious adverse events have been recorded after ingestion of carrot except for some cases of photosensitivityAsri Werdhasari (2014).

Paracetamol (acetaminophen) is one of the most popular and widely used drugs for the treatment of pain and fever. It occupies a unique position among analgesic drugs. Unlike NSAIDs it is almost unanimously considered to have no anti-inflammatory activity and does not produce gastrointestinal damage. Paracetamol is contained in many preparations, available as both over-the-counter and as prescription-only medications. Because of its wide availability paired with comparably high toxicity, (compared to ibuprofen and aspirin) there is a much higher potential for overdose. Paracetamol is especially dangerous on the liver if taken over long periods[3]. If toxic levels of paracetamol occur in the liver, the natural antioxidant defenses of the body are overwhelmed, and the liver is damaged by the buildup of dangerous free radicals. Although nephrotoxicity is less common than hepatotoxicity in paracetamol overdose, renal tubular damage and acute renal failure can occur even in the absence of liver

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 100-105 www.ijprajournal.com ISSN: 2249-7781

injury, and can even lead to death in humans and experimental animals,. Even at normal NSAID dosages, people with compromised kidney function can develop NSAID toxicity. Nonprescriptive analgesic and antipyretic agent has been used widely paracetamol (Bunchorntavakuland Reddy, 2013).

Paracetamol toxicity is one of the major causes of poisoning worldwide, and its overdose is commonly associated with hepatic and renal damages (Placke et al., 1987). Paracetamol toxicity is mediated by the activity of its reactive metabolite known as N-acetyl-p-benzoquinoneimine (NAPQI), which is detoxified by intracellular glutathione (GSH). Therefore, an overdose of paracetamol will saturate the conjugation pathways of GSH and cause depletion of cellular GSH. This subsequently led to a reduced capacity of GSH to detoxify NAPQI. Increased level of NAPQI mediates oxidative damage, and thus enhances cellular injuries and organ dysfunction, including renal damage (Hart et al., 1994).

Although the occurrence of nephrotoxicity is less common than that of hepatotoxicity as reported by researcher, a number of studies have documented that paracetamol-induced renal tubular damage and acute renal failure can occur even in the absence of liver damage, and can be the primary manifestation of paracetamol toxicity. As paracetamol overdose is shown to mediate severe renal damage, which can be life-threatening, antidotes or treatments that could offer maximum protection against paracetamol-induced renal injury are therefore required. To date, clnicianhas been used N-acetyl-cysteine (NAC) for the treatment of paracetamol toxicity (Dilger and Baker, 2007).

Current evidence suggests that Paracetamol-induced hepatorenal injury is mediated by oxidative damage (Das et al., 2010; Kheradpezhouh et al., 2010). Therefore, the alternative treatments for Paracetamol toxicity could be achieved using a natural compound with antioxidant activity. Daucus carota L.is a root vegetable commonly called as carrot, which is a biennial plant commonly cultivated in Local South Sumatera,Curup City. The most abundant phytonutrients present in carrot are phenolics, polyacetylenes, carotenoids, ascorbic acid, and tocopherol especially betacarotenen. It was documented that this plant had the potentials of hypotension, antifertility, hepatoprotective, antispasmodic, antibacterial,monoamine oxidase inhibition, and cyclooxygenase enzyme inhibition.

Carrort(DaucuscarotaL.), or locally known as Wortel, It hasbetacarotene belonging to the Apiaceae family has been shown to possess a number of biological activities, including anti-cancer Sharifah Sakinah et al., 2007; Abdul et al., 2008), anti-inflammatory (Jaganath and Ng, 2000; Somchit and Shukriyah, 2003), antimicrobial (Abdul et al., 2008; Kaderi et al., 2010), and antioxidant properties (Ruslay et al., 2007). Carrot has been shown to contain betacarotene and flavonoid compounds that exhibit the antioxidant properties (Pietta, 2000), of the plants has been shown to exhibit strong antioxidant activities (Ruslay et al., 2007). In a recent study, betacarotene, which is the active compound of the Carrot rhizome, has been shown to protect against paracetamol-induced renal dysfunction extracxtpreserving antioxidant (Ibrahim et al., 2010).The prophylactic action of betacarotene was observed in rats with acetaminophen induced hepatotoxicity. However, the effect of betacarotene on acetaminophen induced nephrotoxicity had not yet been studied. The present study was therefore designed to investigate protective effects of betacarotene against acetaminophen induced nephrotoxicity.In this study, we determined the potential protective effects and antioxidant activities of betacarotene against Paracetamol-induced nephrotoxicity. However, so far, no scientific validity has been made to establish it as a nephroprotective agentbetacarotene.Hence, in the present study, an effort has been made to reduce uric acid serum level by using betacarotene against paracetaol-induced nephrotoxicity in male animalrats.The effects were determined by measuring the levels of uric acid (indicator of renal function),

II. MATERIALS AND METHODS

1 Betacarotene extraction

Fresh rhizomes (1 kg) of carrot were collected from Curup, and authenticated by a plant at Department of Botany Garden, Palembang, and were deposited as a voucher specimen. The specimen was cleaned and chopped into small pieces and then air-dried at room temperature before used (Mangunsong et al., 2019)

2. Paracetamol was procured as marketed formulation Indofarma. Allopurinol was procured as marketed formulation kimiafarma . The diagnostic kits for the estimation of uric acid (Lot no) were obtained Check Uric Test Kit.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 100-105 www.ijprajournal.com ISSN: 2249-7781

3. Experimental protocol

All procedures involving the use of laboratory animals were reviewed and approved by the Animal Ethics Committee of Local Institution. Twenty four male Sprague-Dawley rats (130–220 g) were obtained from the Local Animal Resource Unit. The animals were housed in a controlled environment with room temperature and a 12-h light-dark cycle. Animals were fed mouse pellet and fresh water ad libitum for a week prior to experiments. Rats were randomly divided into six groups containing 4 animals each and all treatments were given daily for ten days. Paaracetamol and betacarotenewas administered orally.Phytochemical screening of the betacarotene was done. Twenty four adult male rats were divided into six groups (n= 4 in each group). Group A (control negatif) animals received feed normal for ten days, group B (paracetamol group) received allopurinol 1,8 mg/200gBB, and paracetamol single dose/day for ten days 300 mg/200gBB orally. Group C received paracetamol 300 mg/200kgBB, Group D received betacarotene 2 mg/200gBB and paracetamol 300 mg/200kgBB, Group E received betacarotene 4 mg/200gBB and paracetamol 300 mg/200kgBB, and group F received betacarotene 8 mg/200gBB and paracetamol 300 mg/200kgBB. respectively, for ten days consecutive of administration. Blood samples withdrawn from the tail to check for uric acid level examination using auotocheckkit tools on day 0, 7^{th} and 10^{th}.All animals were weighed and observation.

Sample preparation

Blood was obtained via tail of rat before and after 10 days of treatment. Blood was apply to the check test andtested for serum level of uric acid .

Statistical analysis

Statistical analysis was done using SPSS version 17.0. Data were analyzed using Shapiro-Wilk normality test and one way analysis of variance (ANOVA) was used for comparison between groups followed by post-hoc Tukey test. Data were expressed as mean±standard deviation (SD) and $P<0.05$ showed statistically significance.

III. RESULT

Preliminary Phytochemical Analysis

A preliminary phytochemical analysis was carried out for the qualitative identification of phytoconstituents in Daucuscarota.

Paracetamol-Induced Nephrotoxicity

Twenty-four healthy SD rats of male sex were weighed and grouped randomly into six groups (n = 4). Group 1 served as normal controlreceiving normal feed and 0.5% carboxymethyl cellulose (CMC) orally for 10 days, group 2 receiving allopurinol 1,8 mg/200gBB and paracetamol doses 300 mg/200gBBand 0.5% CMC (p.o.) for 10 days, whereas group 3 considered as paracetamol-intoxicated group receiving paracetamol doses 300 mg/200gBB/dayand 0.5% CMC (p.o.) for 10 days. Group 4 (DC2) group 5 (DC 4) and group 6 (DC 8) were considered as treatment groups that received DC at doses of 2 mg/200g/day,4 mg/200g/day and 8 mg/200g/day (p.o.), respectively, an hour before paracetamol intoxication for a period of 10 days.

Evaluation of Nephroprotective Potential

After 10 days of experimental protocol, the animals sample blood was collected form the tail. Then, the blood was made to autocheck uric acid by leaving them for 5-7 seconds and it was appear and display the uric acid level by diagnostic kits test. The measurement start from day 0^{th}, 7^{th} and 10^{th}.

Change in body weight

At the final day of the experimental procedure, the body weights were measured to evaluate the change in the body weight from the initial body weight and until after the blood collection, the body were weight to assess the change in weight among the experimental groups.

Extraction

The carrot 500 Gram were cut to small peace, mixer to get fresh juice. Then, filter whatman paper No 1. After filtration, add some $CaCO_3$ and it was centrifuge 3500 rpm 15 minutes by using centrifuge. Remove pellet and supernatant. Pellet remove from water by rotary vacuum evaporator at a temperature not exceeding 40°C to get crude extract betacarotene. The yield was found to be 0,19% w/w.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 100-105 www.ijprajournal.com ISSN: 2249-7781

Tabel 1. Serum Blood Level of Uric Acid and Effect of Betacarotene (Daucuscarota L). on serum levels of in paracetamol -induced nephrotoxicity in SD rats

No	Weight	Control	Allopurinol	Paracetamol	BC2	BC4	BC8	Day
		Uric Acid Parameter mg/dL						
1	**152± 8,5	3,37± 0,37	3,20± 0,61	3,43± 0,22	3,45± 0,66	3,92± 1,3	3,44± 0,66	0
2	*155± 7,10	3,45± 0,37	*8,40± 0,61	**10,83± 0,33	*7,85± 0,66	*5,92± 1,75	*6,6± 2,55	7
3	**164± 6,4	3,40± 0,21	*6,50± 0,61	**12,30± 1,83	*7,85± 0,66	*6,70± 0,60	*6.10± 1,02	10

Value are expressed as mean± SD, *P<0.05 ,[a]compare to Allopurinol, [b]Compare to Paracetamol, BC = Betacarotene, SD=Standard Deviation.

IV. DISCUSSION

Paracetamol has been used widely in all of age in the world to prevent pain and to cure fever. Despite the use of betacarotenewas limited with nutrient , it becomes a promising therapeutic due to its potent nephroprotective properties. Accumulation of drug tends to be a primary key pathological event in paracetamol -inducing nephrotoxicity and subsequent renal dysfunction.Being as polyenene in nature, betacarotene has a strong affinity toward negatively charged brush-border membrane components of proximal tubule where it forms drug receptor complex with megalin, a cationic drug receptor. Then, pinocytosis translocates the drug to lysosomes, where phospholipidosis takes place to interrupt various intracellular renal functions leading to renal injury.This renal injury in turn manifests the migration of monocytes and macrophages to the site of injury by stimulating intercellular adhesion molecule-1 and monocyte chemoattractant protein while several other studies reported the role of reactive oxygen species in implicating the pathogenesis of gentamicin-induced nephrotoxicity.

Drugs such as paracetamol became the major implicating factor in the acute kidney injury due to indigenous functions of kidney to excrete them. This acute renal injury often leads to renal failure which in turn associated to other pathological manifestations such as nephritis, cardiovascular disorders, and diabetes.

Paracetamol are clinically effective as analgesic in the treatment of fever, but they more commonly implicate the nephrotoxicity by free radical generation, loss of brush-border integrity, acute tubular necrosis, and glomerular congestion, which ultimately leads to reduced glomerular filtration rate and acute renal dysfunction.[However, several pharmacological interventions such as antibiotics, calcium channel blockers, beta blockers, iNOS inhibitors, nitric oxide precursors, antianginal, hormones, antiplatelet, statins, peroxisome proliferator-activated receptor gamma agonists, tumor necrosis factor alpha synthesis inhibitors, biguanides, antioxidants, and super oxygen dismutase mimetics had the potential to halt the progression of gentamicin-induced nephrotoxicity, but their clinical setting needs to be debated.

Paracetamol -intoxicated nephrotoxicity is functionally evident by the elevated serum levels of urea, uric acid, and creatinine; structurally characterized by tubular necrosis,. Similar sort of alterations were observed with the allopurinol treatment in paracetamol and treatment groups. As an indicative of decrease in uric acid level

The serum uric acid levels were found to be increased because of accumulation by the decrease in glomerular filtration rate in paracetamol -intoxicated rats, but with the supplementation doses of betacarotene, it dose dependently ameliorated the paracetamol -induced elevated serum levels of uric acid, and creatinine in BC 2 BC4 and BC8 groups. However, in BC 4 group, the nephroprotective property was found to be very prominent with high dose when compared with BC2 group. These results notified the improved renal function by the effective clearance uric acid.

Paracetamol seems not induced weight loss resulted from to a great extent by BC treatment. The increased ofweight of paracetamol -treated rats due to feeding even normalized by the DC treatment. After the paracetamol administration, the paracetamol groups showed the extensive increase uric acid serum level. BC

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 100-105 www.ijprajournal.com ISSN: 2249-7781

supplementation showed the marked attenuation of that even implications that uric acid induced by paracetamol.. Thus, the results demonstrated the ablated inflammatory events by the cellular anti-inflammatory properties of polyacetylenes-betacarotene present in carrot.

By considering the oxidative stress in the pathophysiology of nephrotoxicity, antioxidants therapy opted to be an alternative choice in its management. Polyphenolic compounds reported to possess nephroprotective property by promoting antioxidant enzyme system, thereby attenuating ROS generation and lipid peroxidation. In evidence of this, the polyacetylene compounds of BC can contribute to nephroprotective by its antioxidant activity. The natural antioxidants β-carotene, a terpenoid constituent of the crude extract carrot can ameliorate the nephrotoxicity by its free radical scavenging activity. As a part from the all of our big research, a little bit the results of biochemically, BC had the ability to halt the paracetamol nephrotoxicity dose dependently by having rich antioxidant and cellular anti-inflammatory property. However, the other mechanisms betacarotene properties need to observed.

V. CONCLUSION

Concurrent administration of BC to a great extent in dose dependently attenuated the uric acid level implications of paracetamol nephrotoxicity. Being as a source of rich carotenoid, and polyacetylene constituents in carrot, the principle mechanism to elucidate the nephroprotective potentiality possibly attributed due to its antioxidant and cellular anti-inflammatory properties.

REFERENCES

[1]. Asri Werdhasari (2014) “Peran Antioksidan Bagi Kesehatan,” Jurnal Biomedik Medisiana Indonesia, 3(2), hal. 59–68.

[2]. Bahrami, R., Ghobadi , A Nasim B., Elham A., 2018, Medicinal Properties of Daucus carota in Traditional Persian Medicine and Modern Phytotherapy.J Biochem Tech (2018) Special Issue (2): 107-114 ISSN: 0974-2328

[3]. Baskoro, B. . (2015) Dasar Pemisahan Kimia ”Cara Mudah Memahami Pemisahan Kimia”. Jakarta.

[4]. Bunchorntavakul, C. dan Reddy, K. R. (2013) “Acetaminophen-related Hepatotoxicity,” Clinics in Liver Disease. Elsevier Inc, 17(4), hal. 587–607. doi: 10.1016/j.cld.2013.07.005..

[5]. Ghozaly, M. R. dan Safitri, E. . (2016) “Uji Aktivitas Antioksidan Ekstrak N-Heksan, Etil Asetat Dan Metanol Dari Varietas Umbi Wortel (Daucus Carota L.) Dengan Metode DPPH (1,1-Difenil-2-Pikrilhidrazil),” Sainstech Farma, 9(2), hal. 13–18.

[6]. Das J, Ghosh J, Manna P, Sil PC. Taurine protects acetaminophen-induced oxidative damage in mice kidney through APAP urinary excretion and CYP2E1 inactivation. Toxicology. 2010;269(1):24–34. doi: 10.1016/j.tox.2010.01.003

[7]. Dilger RN, Baker DH. Oral N-acetyl-L-cysteine is a safe and effective precursor of cysteine. J Anim Sci. 2007;85(7):1712–1718. doi: 10.2527/jas.2006-835. Dilger RN, Baker DH. Oral N-acetyl-L-cysteine is a safe and effective precursor of cysteine. J Anim Sci. 2007;85(7):1712–1718. doi: 10.2527/jas.2006-835.

[8]. Hart SG, Beierschmitt WP, Wyand DS, Khairallah EA, Cohen SD. Acetaminophen nephrotoxicity in CD-1 mice. I. Evidence of a role for in situ activation in selective covalent binding and toxicity. Toxicol Appl Pharmacol. 1994;126(2):267–275. doi: 10.1006/taap.1994.1116.

[9]. Hery Winarsi (2007) Antioksidan Alami & Radikal Bebas. Yogyakarta: KANISIUS.

[10]. Intan Logani, Heedy Tjitrosantoso, A. Y. (2017) “Faktor Risiko Terjadinya Gagal Ginjal Kronik Di RSUP Prof. Dr. R. D. Kandou Manado,” Pharmacon, 6(3), hal. 128–136. doi: 10.35799/pha.6.2017.16831.

[11]. Laurence, D. R. (1966) “Evaluation of Drug Activities: Pharmacometrics.,” Journal of Medicinal Chemistry, 9(3), hal. 452–452. doi: 10.1021/jm00321a067.

[12]. Kheradpezhouh E, Panjehshahin MR, Miri R, Javidnia K, Noorafshan A, Monabati A, Dehpour AR. Curcumin protects rats against acetaminophen-induced hepatorenal damages and shows synergistic activity with N-acetyl cysteine. Eur J Pharmacol. 2010;628(1-3):274–281. doi: 10.1016/j.ejphar.2009.11.027.

[13]. Placke ME, Wyand DS, Cohen SD. Extrahepatic lesions induced by acetaminophen in the mouse. Toxicol Pathol. 1987;15(4):381–387. doi: 10.1177/019262338701500401.

[14]. Goodman, G. (2007) Dasar Farmakologi Terapi. Ed 1. Diedit oleh J. G.Hardman. Jakarta.
[15]. Mangunsong, S. et al. (2019) "Penentuan β-karoten dalam buah wortel (Daucus Carota) secara kromatografi cair kinerja tinggi (U-HPLC)," AcTion: Aceh Nutrition Journal, 4(1), hal. 36. doi: 10.30867/action.v4i1.151.
[16]. Mary Baradero,Mary Wilfrid Dayrit, D. Y. S. (2005) Klien gangguan Ginjal. Diedit oleh M. Ester. Jakarta: Buku Kedokteran Egc.
[17]. Mary J. Mycek, Richard A. Harvey, P. C. C. (2001) Farmakologi Ulasan Bergambar. Edisi 2. Jakarta.
[18]. Purwitasari, R. (2016) "Efek Nefroprotektif Ekstrak Daun Karamunting (Rhodomyrtus tomentosa [Aiton] Hassk.) Terhadap nefrotoksisitas yang diinduksi asetaminofen."
[19]. Ramadhan, A. (2019) "Pendahuluan Menurut Food and Drug Administration (FDA) dosis aman menggunakan paracetamol pda dewasa dan anak yang lebih dari 12 tahun adalah 4 gram / hari . Konsumsi paracetamol dosis toksik sebesar 15 gram akan menyebabkan kerusakan hati dan kerusakan," 1(1), hal. 8–15.
[20]. Riskesdas (2013) "Riskesdas 2013." Badan Penelitian dan Pengembangan Kesehatan, hal. 304.
[21]. Riskesdas (2018) "Riskesdas 2018," Badan Penelitian dan Pengembangan Kesehatan, hal. 198.
[22]. Setiawan Dalimartha (2000) Atlas Tumbuhan Obat Indonesia. Kedua. Jakarta: Trubus Agriwidya.
[23]. Slocum, J. L., Heung, M. dan Pennathur, S. (2012) "Marking renal injury: Can we move beyond serum creatinine?," Translational Research. Mosby, Inc., 159(4), hal. 277–289. doi: 10.1016/j.trsl.2012.01.014.
[24]. Soviana, E., Rachmawati, B. dan Widyastiti, N. S. (2014) "Pengaruh suplementasi β-carotene terhadap kadar glukosa darah dan kadar malondialdehida pada tikus sprague dawley yang diinduksi Streptozotocin," Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition), 2(2), hal. 41–46. doi: 10.14710/jgi.2.2.41-46.
[25]. Tjay, T.H., dan K. Raharja. (2007) Obat-Obat Penting : Khasiat Penggunaan dan Efek-Efek Sampinya. Jakarta: PT. Elex Media Komputindo.
[26]. Wijayakusuma, H. M. H. (2004) Penyembuhan Dengan Wortel. 1 ed. Jakarta: Pustaka populer Obor.