International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 1449-1454 www.ijprajournal.com ISSN: 2456-4494

The Effect of Antioxidant in Vitamin C on the Pharmacokinetic Parameter of Paracetamol in the Male Rabbits (Lepus Nigricollis)

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Submitted: 26-05-2022 Revised: 03-06-2022 Accepted: 07-06-2022

ABSTRACT

As an analgesic and antipyretic medicine, paracetamol is widely used in society.The interaction of paracetamol can influence both pharmacokinetic and pharmacodynamics. In the interaction of pharmacokinetic phase, other drugs can disrupt the process of paracetamol absorption and metabolism.

The change in the gastric emptying can disrupt paracetamol absorption and change its half-life, the time to reach maximum concentration and the area below the concentration curve. The drugs affecting metabolism will change the enzyme activities, consequently, the amount and the types of the produced metabolites will change as well.

Vitamin C is the kind of antioxidant that is widely consumed by society in various doses. The patent drugs of the combination between vitamin C and paracetamol can be found anywhere. Vitamin C also plays a role to trigger paracetamol metabolism through Cyt.1A1 enzyme activities.

Purpose : To know the effect of Vitamin C concurrent administration on the profile of paracetamol pharmacokinetic parameter of the male white rabbits.

Method : True experimental with post test only control group design. 12 male rabbits were adapted with a standard diet for seven days, distributed into two groups of six. G group as the control group, which was only administered with 300mg/kg BW of paracetamol suspension and T group as a treatment group. The rabbits were administered with 300mg/kg BW of paracetamol and 300mg/kg BW of vitamin C. The blood sampling was performed from the ear vena on the 3rd, 5th, 10th, 20th, 30th, 40th, 60th, 90th, 120th, 180th, 240th, 300th, and 360th minutes. The level of paracetamol in the plasma was measured by a UV spectrophotometer where the wavelength was 435nm. The blood data was analyzed by an independent T-test.

Result: The results of this research showed that the speed of paracetamol elimination declined, which means that it got longer. Consequently, the time needed for the drug in the blood to decline into half of it got longer as well. The interaction of 50mg/kg BW vitamin C administered concurrently with 300mg/kg BW resulted in the value of: Ka, Kel,declined and the value of:Tp, $t_{1/2e}$, AUC increased. Statistically, it did not affect the profile of paracetamol Pharmacokinetic parameters ($p>0,05$).

Keywords: Vitamin C, paracetamol, interaction, pharmacokinetic parameter of Ka, Kel, tpmax.,$t_{1/2}$, AUC

I. INTRODUCTION

Paracetamol metabolism mainly occurred in the liver through a process of glucuronidation and sulfation into non toxic conjugate. A small part of paracetamol is also oxidized through P-450 cytochrome enzyme into a toxic metabolite of N-acetyl-p-benzo-quinone imine (NAPQI) (Sharma, C.V, and Mehta V. 2014).

In a normal condition, NAPQI will be conjugated by glutathione into cysteine and mercapturic acid conjugate. When administered with a big amount of doses or there is a glutathione deficiency, NAPQI will cause acute liver necrosis (Sharma, C.V, and Mehta V. 2014).

Approximately 85% paracetamol was excreted as conjugated and free through urine within 24 hours. This elimination will decrease in an individual of >65 years old or with a kidney disorder.

(https://pubchem.ncbi.nlm.nih.gov/compound/acetaminophen#section=Metabolite-Pathways.,2017)

Drug interaction can occur between drugs consumed concurrently with drugs, food, or drinks. The impact of this drug interaction can be beneficial; the improvement of drug effectiveness,

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Volume 7, Issue 3 May-June 2022, pp: 1449-1454 www.ijprajournal.com ISSN: 2456-4494

or disadvantageous causing the decline of drug effectiveness (Katzung, BG., 2018).

The interaction between vitamin C and ibuprofen in the rats will affect ibuprofen absorption by lengthening the time of plasma concentration until maximum, decline the maximum level of ibuprofen in the blood, and affect ibuprofen elimination by lengthening its time (Prabowo, S.D.,et al., 2016).

Administering durian juice, containing carbohydrates and alcohol, to the rats can influence paracetamol absorption kinetic, by lowering the value of Ka and Cpmaks, improving paracetamol Tmaks. On the other hand, paracetamol elimination kinetic lowers the value of Vd, Cl, Ke, consequently improves the paracetamol value of AUC and $T_{1/2}$ (Simaremare, P., et al., 2013).

Interaction between curcumin and paracetamol is suspected because the P-450 1A1 catalyst cycle is resisted by the curcumin (Donatus, 1994), where liver microsomal enzyme plays a role in the paracetamol metabolism (Wilmanaand Gan, 2008).

Vitamin C plays a role as a cofactor in a number of hydroxylation and amidation reactions by transforming electrons to enzymes in which metal ions must be in a reduced condition, and in a certain condition, it acts as an antioxidant. Easily absorbed, 70%-90% of vitamin C is absorbed through the digestive tract, metabolism occurs in the liver. Its excretion is through urine in a full form and its sulfate salt form.

The stability of vitamin C is affected by pH condition, an interaction with other compounds, and it prevents from oxidation (Katzung, BG., 2018).

Vitamin C is mostly found in vegetables and fruits, especially the citrus type. There are many tissues, including blood and leukocytes. The most important benefit is on its high doses and antibacterial, estimated on its antioxidant. Vitamin C also highly stimulates the metabolism process due to its redox system, easy to be reoxidized (Tjay, TH and Rahardja, K, 2007).

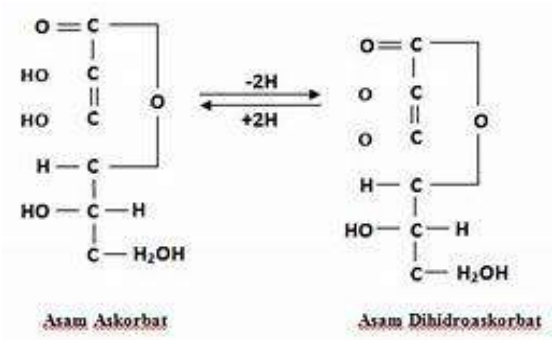

Figure1: Reaction of Vitamin C reduced-oxidation in the body

Vitamin C administration on the guinea pig can improve drug oxidation system in the liver microcosm marked by the improvement of the activities of NADPH cytochrome P-450 reductase, N-demethylase dan O-demethylase (Zannoni and Sato, 1975). Ghiretti and Magaldi (1977), reported that vitamin C can improve the amount NADPH and the activities of liver cytochrome P-450, consequently it improves the drug metabolism.

The administration of vitamin C 50 mg/kg BW of the male rats for 7 consecutive days affects the activities of metabolism and Na diclofenac excretion, Km and Kel improve significantly ($p<0,05$) (Harnis, ZE., 2020).

According to the above consideration, a research studying about interaction between vitamin C and paracetamol, when reviewed from paracetamol pharmacokinetics parameter on the male rabbits, needs to be performed as a benchmark of paracetamol metabolism in the body.

RESEARCH METHOD

The test subjects used in this research are male rabbits which body weight is approximately 1-1,5 kg, aged 3-4 months from the rabbit ranch in Lembang, Bandung. The type is javanese local rabbits (Lepus Negricolis).

The instruments in this research are spectrophotometer, rabbit holder, scalpel, vortex, and centrifugator.

Research procedures

Searching for the time period for the solution to reach fixed absorption

A series of paracetamol levels and water blanks were made; the reading time was started after adding NaOH 10% at 430 nm wavelength.

Searching for the wavelength with maximum absorption

A series of paracetamol levels was made, and then was read at the 390-470 nm wavelengths.

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Volume 7, Issue 3 May-June 2022, pp: 1449-1454 www.ijprajournal.com ISSN: 2456-4494

The making of the standard curve

A series of solutions was made by mixing some paracetamol standard solution with water, adding some reagents account to the implementation of the paracetamol level in the blood. The color intensity was measured at 435 nm wavelength.

Treatment on the experimental animals

12 experimental animals were given ad libitum feeding, divided into 2 groups of 6, before the paracetamol level was determined by Prescot, LF, et al., (1974) method. The experimental animals were fasted. Group I (control) and group II (treatment). Each group was administered with paracetamol suspension of 300mg/kg BW orally. In group II, the rabbits were administered with the treatment of 50mg/kg BW vitamin C orally, together with the paracetamol suspension. On the 3rd, 5th, 10th, 20th, 30th, 40th, 60th, 90th, 120th, 180th, 240th, 300th, and 360th minutes, the blood was drawn through the ear magistralist vena. Samples were processed in plasma form.

Blood sample analysis

The acquired 1,0 ml plasma was added with 1,0 ml TCA 20%, centrifuged at the speed of 3500 rpm for 10 minutes. The liquid was added with 0,5 ml HCl 6 N, 1,0 ml $NaNO_2$ 10%, allowed to stand 2 minutes added with 1,0 ml H_2NSO_3H 15% , added with 2,5 ml NaOH 10 %. Color intensity was measured by spectrophotometerat the maximum wavelength of 435 nm.

The paracetamol level in the blood plasma was calculated based on the acquired standard curve. The calculated pharmacokinetic parameters are $t_{1/2}$ elimination, Ka, Kel, tmax, AUC according to the paracetamol level data in the blood plasma against the time acquired at each group.

Data Analysis

The research data was analyzed statistically using T-test by examining hypotheses to know which group has the same or different effect to one another.

Accuracy

Accuracy in an analysis method is a proximity of the test result value acquired by the procedure using the exact value. This result was considered good because it was based on the literature, the accuracy value was in the range of 80-110% (6) Hall, B, 2008 at Single Laboratory Validation AOAC International, USA: Maryland. Consequently, the method used in this research can be claimed as accurate.

II. RESULTS AND DISCUSSION

The time period determination of paracetamol solution which has stable absorption in this research occurred up to the 50th minute.

The determination of maximum absorption wavelength was acquired at the wavelength of 435nm.

The standard solution curve of paracetamol level was calculated based on the equation of line y = 4,018X + 0,0178, which correlation coefficient was acquired at 0,99968 ($p<0,05$).

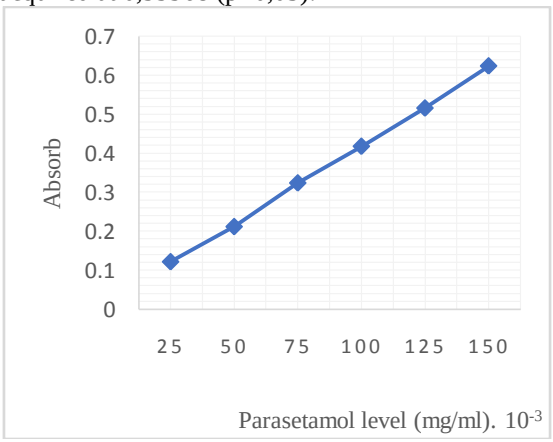

Figure 2 :Paracetamol standard curve: absorption vs parasetamollevel, at the wavelength of 435 nm.

Based on the calculation result,98,05 ± 0,3651% of accuracy value was acquired, consequently, the correlation factor of recovery was 1,020.

As in the table below, the calculation result of the paracetamol pharmacokinetic parameter was based on the equation of line y = 4,018X + 0,0178.

Table. Average value ± SD paracetamol pharmacokinetic parameter before and after treatment

PHARMACOKINETIC PARAMETER	TREATMENT OF PARACETAMOL DOSES 300mg/Kg BW ORAL	TREATMENT OF PARACETAMOL DOSES 300mg/Kg BW WITH VITAMIN C 50mg/Kg BW ORAL

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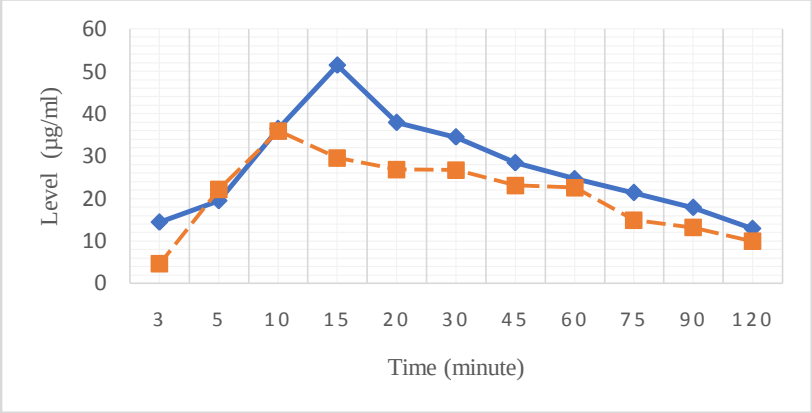
K_a (minute $^{-1}$)	0,091 ± 0,020	0,078 ± 0,019
K_{el} (minute $^{-1}$)	0,005 ± 0,001	0,004 ± 0,001
$T_{1/2}$ (minute)	163,239 ± 29,902	240,650 ± 71,990
Tp (minute)	41,974 ± 8,743	49,336 ± 8,392
AUC (µg minute^{-1})	6699,232 ± 1792,287	7518,046 ± 2664,425

The curve of paracetamol levels in the blood (µg/ml) vs time, before and after administration of vitamin C 50mg/kgBW together with Paracetamol 300mg/kgBW.



Description : before treatment, after treatment

Figure 3 : The curve of paracetamol level in the blood vs the time before and after treatment

Ka value explained absorption kinetics of paracetamol (Hakim L., 2010). Absorption rate depends on the gastric emptying. Paracetamol absorption can be delayed by the food (Moriarty C, Carroll W. 2014). The Ka value in this research dropped to 14,30%.

Kel value in this research dropped to 20%. The decline of Kel parameter was highly affected by the clearance value. The lower the value of clearance, the lower the value of Kel, consequently, it will take longer time for the drugs to be eliminated from the body (Hakim, L., 2010).

Elimination half-life is the time needed by the drug concentration in the blood to reduce by half of the original value. The value of elimination half-life is inversely proportional with Kel, consequently, the lower the value of Kel, the higher the value of $t_{1/2e}$(Hakim, L., 2010., Katzung BG., 2018). In this research, $t_{1/2}$ value experienced a big improvement by approximately 47,40%. At the treatment group, paracetamol Kel became smaller, which means that the paracetamol elimination got longer as well. Consequently, the time needed for the drugs in the blood to reduce into half was longer as well.

Peak plasma concentration showed maximum drug concentration after oral administration (Hakim, L., 2010., Katzung BG., 2018).

AUC is a parameter reflecting the total amount of active drugs to reach systemic cycle. AUC value is directly proportional with Dev and F, but is inversely proportional with Kel and Vd(Hakim, L., 2010). At the trial result, AUC value of the treatment group was higher than the control group.

Tmaks secondary parameter was highly affected by Ka of a drug and not by a drug dose. The smaller the value of Ka, the bigger the value of tmaks, and vice versa (Hakim, L., 2010). Treatment and control group, statistically, the value of Ka did not have a meaningful difference. The Ka between both groups has almost the same big value, consequently, the value of tmaks between both groups also has almost the same value and both of them did not have a meaningful difference.

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Even though there was a reduction by 14,30% of the Ka value, 20% of Kel value, an improvement by approximately 47,40% of $t_{1/2}$ value, 17,50% of tp value, and 12,20% of AUC value, statistically, it did not show a meaningful difference from the t-test result ($p>0,05$).

The administration of an amount of doses can affect the effect of the drugs, including at the pharmacokinetic phase (Hakim, L., 2015). The inability of the vitamin C influences this paracetamol pharmacokinetic parameter, probably because the 50mg/kg BW vitamin C dose could not trigger the activities of cytochrome P-450 of liver microsomal. Consequently, the paracetamol's hydroxylase oxidation process ran normally, as a result, the value of elimination speed constants (Kel) of paracetamol could not be improved and $t_{1/2}$ value could not be reduced.

According to Harnis, ZE, 2020, the administration of 50mg/kg BW vitamin C for seven consecutive days will be able to affect the activities of metabolism and Na diclofenac excretion.

Moreover, the attempted dose of vitamin C could not improve the gastric acidity of the rabbits, consequently, the availability of paracetamol molecule forms could not improve, as a result, the value of absorption speed constants (Ka) remained the same.

It is known that the value of pharmacokinetic parameters can be used for predicting the effect of a drug's pharmacokinetic (Hakim, L., 2015). From this research, it can be stated that the administration of 50mg/kg BW vitamin C together with that of 300mg/kg BW was not likely to improve the effect of paracetamol analgesic.

By another assumption, it can be stated that the interaction of a low-dosed vitamin C combined with paracetamol in the patent medications still plays a role as an antioxidant that helps cure diseases.

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