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The Effect of Avocado to the Profile of Blood Urea Nitrogen (BUN) and Creatinine in Rats (Rattus norvegicus)Induced with Meloxicam

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Abstract

Incidence of drug overdose during treatment of acute disease consequently leads to serious renal damage. As supporting treatment, administration of herbal medicine and food dietary are frequently developed. This research aimed to prove how the administration of avocado juice could lower the BUN and creatinine level in white rats induced with toxic doses of meloxicam. Meloxicam is a non-steroidal anti-inflamatory drug (NSAID), which is effectively used as an anti-inflamatory, analgesic, and antipyretic. Twenty four (24) male Wistar rats were assigned to 4 groups of six rats each (n=6). 1 ml of CMC 1% was administered to Control Group I, 30 mg/kgBB meloxicam and 1 ml of CMC 1% to Control Grup II, 30 mg/kgBB meloxicam and avocado juice 5 g/kgBB/day to Treatment Group I, and 30 mg/kgBB meloxicam and avocado juice 10 g/kgBB/day to Treatment Group II. The study was conducted over 8 days, then the level of Blood Urea Nitrogen and creatinine of the white rats were examined on the 1st and 8th day. The results were analyzed by Anova Two Way With Replication, then followed by T-test (α = 0,05) if there were difference. The Anova Two Way With Replication test showed that the mean of the four groups either the levels of Blood Urea Nitrogen or creatinine was significantly decreased ($p<0,05$). The decrease of BUN in the treatment group I was 27,17 27,17mg/dl and 17,83mg/dl while the decrease of creatinine level was 0,983mg/dl and 0,713mg/dl. The conclusion of this study was that avocado juice decreases level of Blood Urea Nitrogen and creatinine in white rats which exposed toxic doses of meloxicam.
Key words:*Avocado , Blood Urea Nitrogen, Creatinine, Meloxicam, Rats.*

Introduction

The effectiveness and a safety dose is an important issue in clinical medicine (Dheeraj, 2013). It has been many cases reported in dogs and cats regarding the unacurate doses drug, which consume by animals well known as NSAIDs are drugs with the highest rate of consume in the treatment in Europe and the United States (Ulrich, 1999). According FARAD (Food Animal Recidue Avoidance Databank) that the NSAID class of drugs that are included in an extra-label

drugs (drugs with indication unorthodox) include Meloxicam, Aspirin, Carprofe, Flunixin Meglumine, Dipyrone, Ketoprofen, and Phenilbutazon. FDA (Food and Drug Administration) also added a new warning labels that extra-label drug use in purpose of getting information from the vet serious risks associated with extra-label of meloxicam in animals. This label is the result of a FARAD new warning and FDA to identify the number of cases of kidney failure, liver failure and death in cats associated with repeated use of meloxicam (Geof et al., 2008). The pharmacokinetic profile of a new class of NSAID, meloxicam, have been carried out investigations of several species include rats, mice, pigs, and monkeys nation after administration of the drug meloxicam. Overview time plasma concentrations of meloxicam were tested on rats and dogs are very close tightly in humans. The highest concentrations seen in the liver and kidneys. Instead lowest concentration found in the central nervous system (Patty et al., 2016). The effectiveness and safety of a dose is an important issue in clinical medicine (Dheeraj, 2013).

Meloxicam is a new generation of NSAID which can be used in the treatment of arthritis, rheumatism, osteoarthritis and other joint diseases (Livingston, 2000; Papich, 2008). Doses of meloxicam given to dogs should not be > 0.2 mg / kgBW per day whereas for cats it is recommended not more than > 0.1 mg / kgBW per day then slowly the dose is lowered to 0.03-0.05 mg / kgBW (St. Joseph, 2011). Therefore if administration is given more than a normal recommendation, an acute or chronic meloxicam overdose may result in damage to the liver, kidneys and gastrointestinal ulcers. The long-term use of meloxicam without accurate doses can affect kidney function in various ways by inhibiting the synthesis of renal prostaglandins that serve to preserve salt and water homeostasis and to maintain blood flow in the kidneys. Meloxicam nephropathy is characterized by changes in urine volume, glutathione status, creatinine clearance, and increased production of lipid peroxide (Palani et al., 2008; Adeneye et al., 2008) Clinical side effects of using oxycam group drugs ie decreased excretion of sodium, decreased excretion of potassium and decreased renal perfusion. Decreased sodium eczema can cause peripheral edema, hypertension, and usually chronic heart failure (Brater, 1999). One of the most important indices of kidney function is the glomerular filtration rate (GFR), which provides information about the amount of functioning kidney tissue . The most rigorous way to measure GFR is by inulin clearance, but it is rarely used because it involves intravenous infusion with constant velocity and urine reserve at certain times with a catheter. Clinically simple GFR can be measured by BUN and serum creatinine levels (Noer, 2006; Price and Wilson, 2006).

As an effort to prevent the occurrence of kidney damage, can be used additional antioxidants that can fight free radicals in the form of glutathione from outside the body. Avocado is one of the easiest fruit in South Sulawesi (Rukmana, 1997). The avocado glutathione is very high. Avocado fruit contains exogenous antioxidants and some vitamin A, riboflavin (vitamin B2). Riboflavin (vitamin B2) in avocado has an antioxidant effect that acts as a precursor of Flavin Adenine Dinucleotide (FAD), a coenzyme needed by glutathione reductase (Berdanier et al., 2007). The content of avocado glutathione reached 3 times compared with bananas, apples, cantaloupe, and wine (Dorantes, 2006). In addition, it was reported that the leaves of avocado contain castor oil agents that have the ability to antispastic in treating diarrhea (Christian, 2013).

Based on the description already mentioned, meloxicam may cause impaired renal and avocado function as a protective agent against the effects of meloxicam. Treatment and prophylaxis options associated with drug complications are very limited (James et al., 2003). Thus, studies related to meloxicam in the form of impaired renal function becomes imperative. This study aims to determine the effect of avocado protection against renal function disorders caused by meloxicam with indicator BUN and serum creatinine mice.

Materials and Methods

The animals used in this study were 24 male Wistar rats (*Rattus norvegicus*) divided into four groups: 2 control groups (K1 and K2) and 2 treatment groups (P1 and P2). The K1 group was a negative control group was given only 1% Na CMC orally using a mouse cannula on day 0 to day 7, positive control group K2 was given meloxicam solution 30mg / kgBW orally on the first day followed by giving 1% Na CMC up to the seventh day. The P 1 group was given a melamicam solution of 30mg / kgBB on the first day continued on the 2nd day with avocado giving once for 5 days / kg / day per oral for 6 days, and P2 group was given 30 mg / kgBB meloxicam solution on the first day continued on the second day with one avocado feeding 10 g / kg / day for six days.

Rats were in acclimatization at the Hasanuddin University Biopharmaceutical Laboratory for one week and during the study the mice were fed with pedalo with AD II feed code every morning and afternoon, while drink was administered infinitely (*ad libitum*). Preparation of meloxicam of 1 ml of meloxicam solution required 0.01 g of Na CMC powder mixed with 30 mg x weight (rat) (in kg), and dissolved in aquadest to 1 ml. The ingredients used in the treatment group were *Persona americana* avocado fruit bought from Makassar area. The avocado is mashed using a blender and filtered using a tea strainer to produce a smoother one. Avocado was taken 100 g for subsequent divided dose for each treatment ie 5 g / kgBW / day for the first treatment group, and 10 g / kgBW / day for the second treatment group. Route of administration orally using a gastric cannula tool have been evaluated where it is found that the distribution of avocado is easier, preventing stress in animals, and especially preventing avocado fruit can be re-released in a certain amount each time administered. This is done 1x / day, daily, for 7 days. 1% Na CMC solution is made by dissolving 1 g Na CMC into aquadest as much as 100 ml, then taken 1-2 ml, adjusted to the white mouse weight conversion.

On day 0 of K2, P1 and P2 groups were given a meloxicam 30 mg treatment while the K1 group was given only 1% Na CMC. On the 1st day, 1 ml - 2 ml of blood is taken through the heart of the left ventricle for examination of BUN and serum creatinine levels in the experimental animals. After blood sampling, continued with 5 g of avocado fruit in the P1 group and 10g / kgBW in group P2. While in group K1 and K2 given Na CMC 1%. After the treatment period was completed for 7 days, it was fasted for 24 hours and on the 8th day anesthesia was performed on all mice using ether solution, then taken blood from the heart of the left ventricle. This blood collection aims to check the levels of BUN and serum creatinine.

Measurement methods of BUN and serum creatinine used are as follows, blood samples taken 2 times (after the administration of meloxicam and after the treatment of avocado fruit) with each volume of 1ml - 2ml. Each blood sample was placed on a 5 ml plain sample bottle that had been labeled and allowed to stand for 15 minutes. The blood sample was centrifuged for 5 minutes at a rate of 5000 rpm to separate serum from blood cells. After the serum is separated from plasma, the serum is inserted in a newly labeled serum cup. Each cup is inserted into The Advia Chemistry XPT system Siemens Healthcare Tarritown - NY, which is a tool used to measure BUN and serum creatinine levels.

Observations and records were performed on BUN and serum creatinine in male white blood observed at the time of meloxicam administration on day 1 and after treatment of avocado on day 8. The data obtained were treated with Anova Two Way With Replication and if

different were followed by T-Test to see the difference of BUN and serum creatinine levels among the groups from 2 control groups and 2 groups of treatment to avocado fruit effect.

Results and Discussions

The results of BUN dan creatinine level in rats treated by avocado post meloxicam induced toxic dose in group K1, K2, P1 and P2 can be seen in Table 1, Figure 1 and Figure 2. In the Figure 1 is presented a graph that gives an overview of BUN levels for 8 days, while Figure 2 presented a graph that illustrates the profile of creatinine for 8 days. The table shown there is also an average value with a standard error of the mean (SEM) so that it can be seen within the increase or decrease in the results of measurements of BUN and serum creatinine.

Table 1. The average levels of BUN and serum creatinine (mg / dl) in each groups induced by administration of meloxicam and avocado on day 1 and day 8.

Group	BUN levels (mg / dl)		Creatinine levels (mg / dl)	
	Day 1	Day 8	Day 1	Day 8
K1	19.33 ± 0.88	19.5 ± 0,61a	0.575 ± 0.03	0.60 ± 0,034a
K2	45.33 ± 1.68	45.17 ± 1,1b	1.35 ± 0.06	1.38 ± 0,054b
P1	43.16 ± 2.52	27.17 ± 0,9a	1.216 ± 0.07	0.983 ± 0,03a
P2	45.66 ± 2.65	17.83 ± 0,7a	1.267 ± 0.07	0.713 ± 0,06a

The kidneys damage can be caused by excessive doses of meloxicam resulting in elevated levels of BUN and serum creatinine. Kidney disorders are indicated by elevated levels of urea and creatinine that should be excreted through the urine but reabsorbed into the bloodstream so that the levels in the blood increase. According to Schrier (2008), serum creatinine is freely filtered in the glomerulus, not absorbed, but undergoes tubular secretion. Thus, creatinine spending that exceeds inulin clearance, is used as a standard glomerular filtration rate. In contrast, urea is freely filtered, not secreted, but reabsorbed by the renal tubules. Urea reabsorption depends on the flow of urine so that more urea is absorbed at lower urine flow rates. At K1 or control, BUN levels ranged between 19.51 ± 0.61 mg / dl and serum creatinine 0.61 ± 0.034 mg / dl which is the normal range. The normal range of BUN levels in mice was13.9 - 28.3 mg / dl and the normal range of normal creatinine levels in mice was 0.30 to 1.00 (Girindra, 1986).

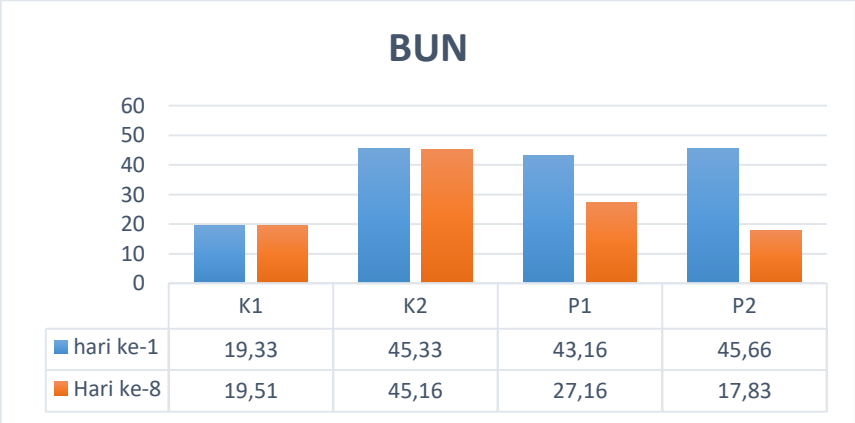

Figure 1. Information showed the same letter of superscripts on the column are not significantly different (p> 0.05)

Levels of BUN on day 1 and day 8 in the positive control group K2 with meloxicam 30 mg / kgBW without avocado fruit continued to show increased levels of 45.17 ± 1.1 mg / dl and serum creatinine levels also increased 1.38 ± 0.054 mg / dl. Increased BUN and creatinine

indicate the occurrence of renal tissue damage due to acute meloxicam accumulation (Schnellman, 2001). The same is also evidenced by research conducted by Ng Lin Eng (2008) which shows a decrease in membrane activity and even necrosis of the mitochondrial membrane in the kidney due to exposure to meloxicam toxic dose in a long time. Similar research also conducted by Al Rekabi (2009) reported that subcronical effects on hematology and histopathology of the liver resulting from toxic dose meloxicam.

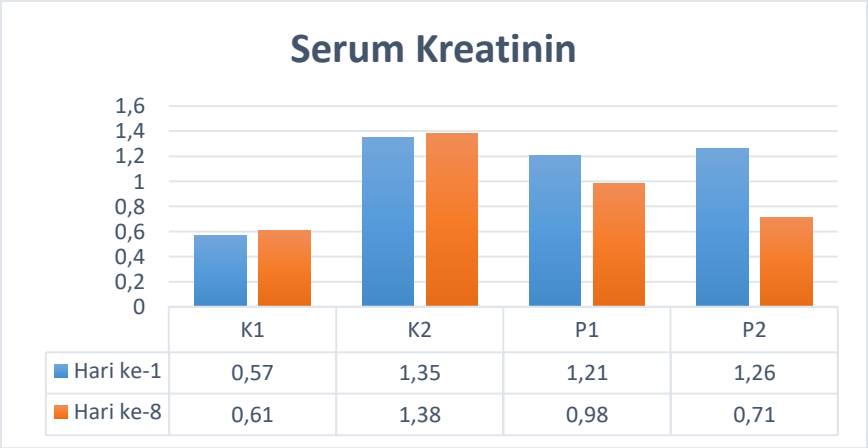

Figure 2. Diagram of the average lavels of creatinine (mg / dl) in each groups induced by administration of meloxicam and avocado day 1 and day 8

The P1 group which was given meloxicam followed by giving avocado fruit dose 5 g, showed the decrease of BUN and serum creatinin from day 1 until day 8 respectively that is 43,16 ± 2,52 become 27,17 ± 0.9 and 1.216 ± 0.07 mg / dl to 0.983 ± 0.03 mg / dl. BUN and creatinine levels were still high compared to the control group but still within the normal range.The P2 group treated with avocado 10g / kgBB showed the highest decrease among all groups. The decrease in levels from day 1 to day 8 is BUN content of 45.66 ± 2.65 mg / dl to 17.83 ± 0.7 mg / dlsedangkan creatinine levels of 1,267 ± 0.07 mg / dl to 0.713 ± 0.06 mg / dl. This indicates that avocado fruit in the P2 group is the optimal dose, which is 10gram / kgBW / day because it is able to repair kidney damage due to toxic doses of meloxicam with a decrease in BUN and creatinine levels close to normal. This proves that the antioxidants in avocado can neutralize the kidney damage due to toxic dose meloxicam with indicator BUN and creatinine levels drop to their normal levels.

Avocado which used contains an antioxidants in the form of precursors of glutathione, namely glutamic acid, glycine, cystine, and methionine. These four amino acids are absorbed through the digestive tract of proteins in the stomach and continue in the small intestine, and enter the blood circulation through the portal vein, then taken to the liver and used as the substance to synthesize glutathione (Almatsier, 2001). Glutathione acts as an antioxidant with several mechanisms, which prevents the reactive oxygen bonds and peroxinitrite with mitochondria, and provides the energy of the mitochondrial substrate (Saito et al., 2010). In addition, glutathione can regenerate the most important antioxidants, namely lipoic acid, vitamin C and E, back to the active form, and also reduce the tocopherol radicals directly and indirectly through the reduction of semidehidroaskorbat into ascorbate (Mohora et al., 2007). Vitamin C can also improve endothelial function by restoring nitric oxide resulting in vasodilation in the endothelium (Barrett and Parfrey, 2006).

In addition to the glutathione precursor content, avocados also have vitamin B2 (riboflavin) that acts as FAD precursors, the coenzymes required by glutathione reductase and mineral in

the form of selenium contain antioxidant effects on glutathione peroxidase enzymes which will support the function of glutathione in body. In Pantun Sagala (2010) study mentioned that the granting of avocado fruit has the power of protection against damage to the gastric mucosa due to exposure to aspirin toxic agents in mice. The ethanol extract of avocado leaf is reported have an effective agent in inhibiting the formation of calcium oxalate crystals in the kidneys. Avocado has the potential of natural antioxidants and anti-inflammatory that can prevent the formation of kidney stones by interrupting the process of destruction of epithelial cells (Ietje et al., 2012).

Conclusion

Based on the results of study, it can be concluded that the of avocado can reduce levels of BUN and serum creatinine in rats induced meloxicam to the normal levels within 7 days of administration. The optimal dose to reduce BUN and serum creatinine serum induced by meloxicam was 10g / kgBW / day.

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