



The Effect of Ajwa Dates Extract (*Phoenix dactylifera L.*) on the Creatinine Levels in White Rats (*Rattus norvegicus*) Induced by Meloxicam Toxic Doses

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Abstract

Meloxicam is an NSAID (non-steroidal anti-inflammatory drug) which is an antipyretic analgesic drug as well as a non-steroidal anti-inflammatory drug that is widely used by the public, including pet owners, in treating their favorite animals without advice from a veterinarian. The use of meloxicam in excessive doses can trigger kidney damage. Based on related research, meloxicam can cause cell death due to nephrotoxicity. Ajwa dates have the highest antioxidant activity among other types of dates, suppress lipid peroxidation, prevent cell damage, and have nephroprotective abilities. The aim of this study was to determine the effect of giving ajwa date extract on creatinine levels of male wistar rats induced by toxic dose of meloxicam. This research is an experimental research with the research design used is the Pre Test-Post Test Design Group. The total sample used was 24 male wistar rats which were divided into 4 groups, namely the negative control group (K1) which was given 1% Na CMC on days 1-18, the positive control group (K2) which was given meloxicam 30mg/kgBW on day 1- 4 and 1% Na CMC on days 5-18, treatment group 1 (K3) was given meloxicam 30mg/kgBW on days 1-4 and ajwa date extract 150mg/kgBW on days 5-18, treatment group 2 (K4) given meloxicam 30 mg/kg body weight on days 1-4 and ajwa date extract 300 mg/kg body weight on days 5-18. Blood sampling was carried out on day 19 to measure creatinine levels. The results of data analysis using the Independent T-Test test for groups K1 and K2 were $P < 0.05$, which means that there is a statistically significant relationship between the administration of meloxicam and the increase in creatinine levels. The results of data analysis using the independent T-Test test for the K2 group with K3 and K4 were $P < 0.05$, which means that there is a statistically significant relationship between the administration of ajwa date extract and the decrease in creatinine levels of rats given a toxic dose of meloxicam.

Key words: Ajwa dates, Creatinin, Meloxicam, Nephroprotective, White Rat

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Introduction

One of the lifestyles of today's urban communities is to keep pets. This is done to reduce work stress and a means of channeling hobbies. The most popular pets are cats and dogs. However, there are still many pet owners who do not have awareness of the health of their pets, such as giving medicines without consulting or direct prescriptions from a veterinarian when their pet is sick. Pet owners often treat their pets, by consuming body temperature-lowering drugs or

inflammation drugs without knowing the right dose and the effects that arise when consumed long-term and with excessive doses on pets. One of them is the use of drugs commonly prescribed by veterinarians at clinics in Makassar, namely meloxicam.

This drug is a new generation of NSAID (non-steroidal anti-inflammatory drug) which is an antipyretic analgesic drug and non-steroidal anti-inflammatory drug that is widely prescribed and also used without a doctor's prescription. According to FARAD (Food Animal Residue Avoidance Databank), the NSAIDs included in extra-label drugs include Meloxicam, Aspirin, Carprofen, Flunixin Meglumine, Dipyrone, Ketoprofen, and Phenilbutazone. The FDA (Food and Drug Administration) has also added a new warning label, namely the use of extra-label drugs in the hope that veterinarians will be informed of the serious risks associated with using extra-label meloxicam in animals. This label is a new warning from Farad and the FDA which identifies many cases of kidney failure, impaired liver function, and death in cats associated with repeated use of Meloxicam (Geof et al., 2008). Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that can be used in the treatment of arthritis, rheumatism, osteoarthritis, and other joint diseases (BNF, 2009). The dose of meloxicam given to dogs should not be > 0.2 mg/kgBW per day while for cats it is recommended that it should not be >0.1 mg/kgBW per day and gradually reduce the dose to 0.03-0.05 mg/kgBW (St. Joseph, 2011). Therefore, if the administration is given more than the normal recommendation, there will be an acute or chronic overdose of meloxicam which can cause damage to several organs, one of which is the kidney.

There are two types of examinations that can be performed to detect kidney disease and evaluate kidney function, namely biochemical methods (urine chemistry examination, glomerular filtration rate and tubular function tests) and morphological methods (microscopic examination of urine, bacteriological examination of urine, radiological examination, and examination of urine). kidney biopsy). 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. Clinically simple GFR can be measured by BUN (Blood Urea Nitrogen) and serum creatinine level (Noer, 2006; Price and Wilson, 2006).

To prevent kidney damage due to meloxicam overdose, additional antioxidants can be used to fight free radicals in the form of glutathione from outside the body. However, glutathione levels cannot be increased to clinically beneficial levels through oral single-dose glutathione consumption because production in cells requires the amino acids glycine, glutamate and cystine for precursors. One of the plants that contain antioxidants is dates (*Phoenix dactylifera L.*) which contain compounds that can reduce the effects of free radicals on the fruit and seeds (Baliga et al. 2011). Dates are a good source of phytochemicals, including carotenoids, phenolics, and flavonoids. Dates can not only provide antioxidant, antimutagenic, and immunomodulatory benefits for health but also have diverse medicinal values, including antihyperlipidemic, anticancer, gastroprotective, hepatoprotective, and nephroprotective properties (Tang et al. 2013).

The results of research conducted by Ramadhan et al. (2019) explained that date palm extract (*Phoenix dactylifera*) has effectiveness as a nephroprotector. Based on this description, a study was conducted on the effect of giving ajwa date fruit extract on the creatinine picture in white rats (*Rattus norvegicus*) after administration of a toxic dose of meloxicam.

Materials and Methods

This research was conducted at the Veterinary Clinic of the Medical Faculty of Hasanuddin University in June-October 2021. The manufacture of ajwa date extract was carried out at the

Biopharmaceutical Laboratory of the Faculty of Pharmacy Unhas using the maceration method. This study used 24 male white rats (*Rattus norvegicus*) wistar strain which were divided into positive control group, negative control group, treatment 1, and treatment 2.

The study was conducted by dividing the sample into the control group and the treatment group through randomization. This design was expanded to involve more than one independent variable, in other words, the treatment was carried out on more than one group with different forms of treatment. After all treatments were completed, observations (post tests) were carried out in all groups to obtain conclusions about the differences between them through certain data analysis (Notoatmodjo, 2005).

The research design used was the Pre Test-Post Test Design Group. The design was chosen with the assumption that within a certain population, each population unit is homogeneous, i.e. the characteristics between all population units are the same. This design was chosen because in this study, serum creatinine measurements were carried out in both the control group and the treatment group.

The 24 male wistar rats that had been adapted for one week were divided into 4 groups, namely 2 control groups and 2 treatment groups. The K1 group was a negative control group that was only given 1% Na CMC on day 1 to day 18, the positive control group K2 was given 30mg/kgBW meloxicam solution on days 1-4 followed by administration of 1% Na CMC until day 5- 18. The K3 group was given a 30mg/kgBW meloxicam solution on days 1-4 followed by 150mg/head/day ajwa date extract on 5-18 days, and the K4 group was given 30mg/kgBW meloxicam solution on days 1-4 followed by administration Ajwa date extract as much as 300 mg/head/day 5-18 days.

On the 19th day, 1ml-2ml of blood was drawn to check serum creatinine levels in experimental animals. Blood collection was performed under anesthesia on all rats using zoletyl anesthesia, then the blood was taken from the eye veins using a microcapillary tube and then inserted into the EDTA tube for separation from serum and continued by checking creatinine levels.

The data obtained are processed and will be tested for normality, if the data are normally distributed, it will be followed by an independent T-Test test to see the difference in serum creatinine levels between the groups of 2 control groups and 2 treatment groups on the effect of ajwa date palm extract.

Results and Discussion

Based on the results of the study, the following data were obtained:

Table 1. Independent T-Test results for the negative control group (K1) and the positive control group (K2)

Group	n	Average Osteocyte	*p
K1	6	0.82	0.00
K2	6	4.87	

K1 = Negative control group, not given meloxicam

K2 = Positive control group, given meloxicam 30mg/kgBW

*P = Independent T-Test

The results of the Independent T-Test showed that the P value was below 0.05, namely P = 0.00, which means that there is a statistically significant relationship between the administration of meloxicam and the increase in creatinine levels. The average creatinine level in the positive control group (K2) was 4.87mg/dl, a significant increase compared to the negative control group (K1), which was 0.82mg/dl. The average creatinine level of the K2 group was above the normal level of rat creatinine. The normal levels of creatinine in wistar rats ranged from 0.578 to 1.128 mg/dl. This shows that meloxicam can interfere with kidney function by looking at serum creatinine levels that are above normal after meloxicam administration. Creatinine is one indicator to see kidney function. Meloxicam can affect kidney function in various ways by inhibiting renal prostaglandin synthesis, which functions to maintain salt and water homeostasis and to maintain blood flow to the kidneys. The clinical side effects of using the oximam class of drugs are decreased sodium excretion, decreased potassium excretion and decreased renal perfusion.

The results of the study using the isolation of kidney mitochondria, concluded that meloxicam is toxic to kidney cells by interfering with the function of mitochondria. The meloxicam was shown to act as uncouplers by scattering mitochondrial membranes and inhibiting ATP biosynthesis due to its ability to inhibit aspartate thereby limiting the bioavailability of the two main substrates glutamate and malate. Due to decreased mitochondrial function, it is not surprising that meloxicam can cause nephrotoxic cell death.

Creatinine is a metabolic product of creatine and phosphocreatine. Creatinine has a molecular weight of 113-Da (Dalton). Creatinine is filtered at the glomerulus and reabsorbed tubularly. Plasma creatinine is synthesized in skeletal muscle so its levels depend on muscle mass and body weight (Alfonso, 2016). The initial process of creatine biosynthesis takes place in the kidney involving the amino acids arginine and glycine. According to one in vitro study, creatine is converted to creatinine in the amount of 1.1% per day. In the formation of creatinine there is no reuptake mechanism by the body, so most of the creatinine is excreted through the kidney. If renal dysfunction occurs, the creatinine filtration ability will decrease and serum creatinine will increase. A twofold increase in serum creatinine level indicates a 50% decrease in renal function, as well as a threefold increase in serum creatinine level reflects a 75% decrease in renal function. There are several causes of increased creatinine levels in the blood, namely dehydration, excessive fatigue, use of drugs that are toxic to the kidneys, kidney dysfunction with infection, uncontrolled hypertension, and kidney disease (Nicola, 2012).

Table 2. Independent T-Test results positive control group (K2) with treatment group 1 (K3)

Group	n	Average Osteocyte	*p
K2	6	4.07	0.00
K3	6	1.03	

K2 = Positive control group, given meloxicam 30mg/kgBW

K3 = The treatment group was given meloxicam 30mg/kgBW and ajwa date extract 150mg/kgBW

*P = Independent T-Test

The results of the independent T-Test showed that the P value was below 0.05, namely P = 0.00, which means that there is a statistically significant relationship between the administration of Ajwa date extract and the creatinine levels of rats given a toxic dose of meloxicam. The average creatinine level of rats given meloxicam (K2) was 4.07 mg/dl, a very significant decrease in treatment group 1 (K3), namely the administration of ajwa date extract

150mg/kgBW with an average creatinine level of 1.03mg/dl which was in the range Wistar rats' normal creatinine values. This shows that the administration of ajwa date extract can reduce creatinine levels, which means that ajwa date palm extract can improve kidney function damaged by toxic doses of meloxicam. In addition, the results of the independent T-Test for K2 with K3 and K4 is $P = 0.00$ which means statistically.

Table 3. Independent T-Test results positive control group (K2) with treatment group 2 (K4)

Group	n	Average Osteocyte	* <i>p</i>
K2	6	4.07	0.00
K4	6	1.35	

K2 = Positive control group, given meloxicam 30mg/kgBW

K4 = The treatment group was given meloxicam 30mg/kgBW and ajwa date extract 150mg/kgBW

**P* = Independent T-Test

The results of the Independent T-Test showed that the *P* value was below 0.05, namely $P = 0.00$, which means that there is a statistically significant relationship between the administration of Ajwa date fruit extract and the creatinine levels of rats given a toxic dose of meloxicam. The average creatinine level of rats given meloxicam (K2) was 4.07 mg/dl, a very significant decrease in treatment group 2 (K3), namely the administration of ajwa date extract 300mg/kgBW with an average creatinine level of 1.35 mg/dl although the levels were still small. above normal levels of rat creatinine. The normal range of creatinine in wistar rats is 0.578-1.128 mg/dl. This shows that the administration of ajwa date extract can reduce creatinine levels, which means that ajwa date palm extract can improve kidney function damaged by toxic doses of meloxicam.

Various studies have shown that *Phoenix dactylifera L.* contains antioxidant (El Arem et al., 2014), gastroprotective (Souli et al. 2014; Yasin et al. 2015), hepatoprotective (Abdelaziz and Ali, 2014; Saafi et al. 2011), nephroprotective (Khalid et al. 2017; Saafi-Ben Salah et al., 2012; Sahyon and Al-Harbi, 2020; Yasin et al. 2015), and has anti-cancer activity (Khan et al. 2017; Yasin et al. 2015).

The results of the study that explain the efficacy of dates are research conducted by Al-Erem et al. (2014), the results of the research conducted explain that date palm extract (*Phoenix dactylifera*) has effectiveness as a nephroprotector against dichloroacetic acid-induced nephrotoxicity in adult rats. The administration of dichloroacetic acid in rats caused an increase in malondialdehyde levels in the kidneys, but the administration of date fruit extract decreased malondialdehyde levels, decreased urea and creatinine levels through the capacity of dates as antioxidants. In addition, Jamila (2015) in his research said that the date palm extract had a nephroprotective effect on exposure to rhodamine B by finding an improvement in the glomerulus and kidney tubules. This study also concluded that the nephroprotective ability of dates against kidney damage is due to the high antioxidant effect of dates.

Ajwa dates have been shown to maintain the highest antioxidant activity among other types of dates, suppress lipid peroxidation, prevent cell damage, improve cancer therapy and reduce side effects caused by conventional chemotherapy (Sahyon and Al-Harbi, 2020), as antiviral, antifungal, anti-bacterial, anti-inflammatory, anti-diabetic, and many more benefits from Ajwa dates (Khalid et al. 2017).

Dates (*Phoenix dactylifera L.*) have a strong antioxidant effect due to their high content of coumaric and ferulic acids, flavonoids, phenolic compounds, and anthocyanins. Several in-vitro studies and in-vivo studies, demonstrated the strong antioxidant properties of date fruit and date seed extract (Alghamdi et al. 2020). Fresh dates contain high levels of antioxidants, anthocyanins, carotenoids, phenolics, free and bound phenolic acids (Abdu, 2018).

Inflammation is one of the important physiological defense mechanisms against various factors such as infection, burns, toxic chemicals, allergens and other stimuli (Sharma et al, 2011). An unbalanced inflammatory process shows an important role in the development and progression of various diseases. The transcription factors LOX and NF-kB play important roles in inflammation, cancer, diabetes and other diseases. The regulation of transcription factors is an important and critical step in disease prevention. Transcription factor inhibitors show an important role in preventing the action of transcription factors. Unfortunately, the inhibitors used today show side effects and are also expensive. Natural products are good drugs in suppressing NF-kB and act as anti-inflammatory agents. Previous studies have shown that plant constituents such as phenolics and flavonoids act as excellent anti-inflammatory agents (Zhang et al. 2013).

Dates have an important anti-inflammatory role and a recent report on Ajwa dates showed that ethyl acetate, methanol and water extracts from Ajwa dates inhibited lipid peroxidation of the COX-1 and COX2 cyclooxygenase enzymes (Thaulhok et al. 2007). An animal model study showed that Phoenix dactylifera extract had a potential protective effect through modulating cytokine expression (Elberry et al 2011). Another important finding in support of the date palm fruit was reported that the methanol extract of the edible part of the fruit showed an important role in reducing leg swelling and plasma fibrinogen (Muhammad, 2004). A study that supports dates as anti-inflammatory shows that dates can be considered a good source of natural antioxidants and anti-inflammatory drugs (Eddine, 2004). A study of Phoenix dactylifera flesh extract in a rat model of nephrotoxicity showed it significantly reduced the induced increase in plasma creatinine and urea concentrations and ameliorated proximal tubular damage (Al-Karawi et al. 2008).

Conclusion

Based on the results of the research conducted, it was concluded that the administration of toxic doses of meloxicam to male wistar rats could interfere with kidney function by observing a significant increase in serum creatinine levels after meloxicam administration. Creatinine is one indicator to see kidney function. Meloxicam can affect kidney function in various ways by inhibiting renal prostaglandin synthesis, which functions to maintain salt and water homeostasis and to maintain blood flow to the kidneys. The clinical side effects of using oxycam class of drugs are decreased sodium excretion, decreased potassium excretion and decreased renal perfusion. In addition, administration of ajwa date fruit extract can significantly reduce creatinine levels, which means that ajwa date palm extract is able to improve kidney function damaged by meloxicam. toxic dose. The dose of ajwa date fruit extract that showed the best results in reducing creatinine was a dose of 150mg/kgBW with an average serum creatinine of 1.03 mg/dl.

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