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Effect of Ajwa Date Fruit Extract (Phoenix Dactylifera L.) Against Liver Damage in White Rats (Rattus Norvegicus) Induced by Meloxicam

Fatimah Mappanyompa[a], M Aryadi Arsyad[b*], Dwi Kesuma Sari[c], Sartini Natsir[d], Muhammad Husni Cangara[e] Yulia Yusrini Djabir[d]

[a]Master of Biomedical Science, Postgraduate School Hasanuddin University, Makassar, Indonesia
[b]Department of Physiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[c]Study Program of Veterinary Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[d]Faculty of Phamacy, Hasanuddin University, Makassar, Indonesia
[e]Department of Histology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

*corresponding author: email: fatimahmapp@gmail.com
Contact Person: 082194759721. Jl. Perintis Kemerdekaan km.10, Tamalanrea, 90245

Abstract

Objective: This study was aimed to investigate the protective effect of Ajwa date palm extract (Phoenix Dactylifera L.) against liver damage in white rats (Rattus norvegicus) induced by Meloxicam. **Methods:** The samples used in this study were twenty five male white rats divided into five groups with body weight 200 – 300gr, namely a healthy control group, negative control given Meloxicam 30mg/kgBB, treatment group 1 was given Ajwa date fruit extract 75mg/kgBW and Meloxicam 30mg/kgBW, treatment group 2 was given Ajwa date fruit extract 150mg/kgBW and Meloxicam 30mg/kgBW, and treatment group 3 was given Ajwa date fruit extract 300mg/kgBW and Meloxicam 30mg/BW for 14 days. On the 15th day, the rats were anesthetized for blood tests and euthanized for liver organ observation. **Results:** The result showed by giving Ajwa date palm extract with higher doses and Meloxicam 30mg/kgBW, resulted a decrease in ALT and AST enzyme levels which were close to normal values in rats. Histological observation showed extensive damage to liver cell tissue, with signs of inflammation, hemorrhage, necrosis, and hydropic degeneration. By giving high doses of Ajwa date palm extract and Meloxicam 30mg/kgBW, gave a view of a decrease degree on liver tissue damage. **Conclusion:** The extract of Ajwa date palm (*Phoenix Dactylifera L.*) can provide a protective effect against liver damage in white rats (*Rattus Norvegicus*) induced by Meloxicam with decreased levels of liver function enzymes which are *Alananine Transaminase* (ALT) and *Aspartate Aminotransferase* (AST), along in the changes of liver histopathological view which showed a decrease degree of liver cell tissue damage.

Keywords: Ajwa date palm extract; Meloxicam; Liver tissue damage.

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Introduction

In the last 10 years, research involving dates (Phoenix dactylifera L.) has been widely studied by researchers in the fields of medicine, biomolecules, and pharmaceuticals as a preventive effect

against various diseases, exposure to chemical compounds or organophosphates such as insecticides which consequently generate free radicals (Saafi-Ben Salah et al., 2012). Ajwa dates have been shown to have 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).

Liver or liver disease caused by ingestion of large quantities of NSAIDs (Non Steroid Anti Inflammatory Drugs), despite the low overall incidence rate of NSAID-induced hepatotoxicity, their widespread use is an important cause of drug-induced liver damage (Sriuttha et al., 2018).

Meloxicam is one of the NSAIDs (Non Steroid Anti Inflammatory Drugs) / NSAIDs (Non Steroid Anti-Inflammatory Drugs) which are widely used to reduce inflammatory activity, reduce pain, reduce fever, swelling and increase mobility in the incidence of arthritis and other conditions such as rheumatoid arthritis. arthritis, osteoarthritis, and acute ankylosing spondylitis arthritis (Brogden et al., 1980). In a prospective study it was stated that up to 7% of patients taking Meloxicam had elevated liver enzymes, and the severity of liver damage from meloxicam ranged from elevated liver enzyme levels without symptoms to symptomatic hepatitis with or without jaundice (Betnesda, 2020).

Based on the description above, the researchers were interested in seeing whether there was a protective effect of giving ajwa date fruit extract against liver damage in white rats (Rattus norvegicus) induced by Meloxicam.

Meterials and Methods

Research Permit and Ethical Eligibility.

The research process was carried out by following the *Guide for the Care and Use of Laboratory Animals, Edition 8, by the Institute for Laboratory Animal Research 2011* and approved by the ethical commission of the Faculty of Medicine, Hasanuddin University (Number: 187/UN4.6.4.5.31/PP36/2022)

Population and Sampling Technique.

Twenty five male Rattus norvegicus white rats weighing 200-300g were divided into five groups (n = 5). Subjects were treated in a laboratory controlled for temperature, air pressure, humidity, dark and light cycles every 12 hours, given standard feed and drink.

Tools and Materials.

Tools: Ajwa date fruit extraction tool, iChem-535 blood chemistry machine, Olympus X5201 microscope, EDTA tube, water bath, hot plate, paraffin, and an oven to dry Ajwa dates. Ingredients: 1% Na CMC solution, 10% neutral buffered formalin, 90% alcohol, 80% alcohol, 70% alcohol, acid alcohol, aquadest, hematoxylin, and eosin.

Ajwa Date Extract and Meloxicam

In the healthy group, neither Ajwa date extract nor Meloxicam was given, the negative group was given Meloxicam on days 1-14. Treatment groups 1, 2, and 3 were given Ajwa date extract at doses of 75, 150, and 300 mg/kgBW and Meloxicam 30mg/kgBW was given on days 1-14. On the 15th day, the mice were anesthetized for blood examination and euthanized for liver organ histopathological observation.

ALT, AST, and Liver Histopathology Examinations.

The ALT and AST measurement were used by taking 2-3 ml blood samples using an EDTA tube. Blood samples were centrifuged for 5 minutes at a speed of 5000 rpm. The blood samples were

analyzed with the iChem-535 blood chemistry machine. The process of making histological preparations in which each group was euthanized under anesthesia using a combination of ketamine-xylasin was then followed by abdominal surgery to remove the liver organ. The fixed specimens were then dehydrated in graded alcohol, clarified in graded xylol, embedded in paraffin, cut into ±5 m thin strips, and stained with hematoxylin and eosin (H&E). Observation of changes in liver histology was observed under a microscope.

Results and Discussion

The results of statistical analysis showed that in healthy controls, ALT and AST levels increased which were still within the normal limits, namely 21.62 IU/L and 63.28 IU/L. Compared with the negative control (Meloxicam 30mg/kgBW) given for 14 days, the results of ALT levels increased to 60.28 IU/L and AST 120.9 IU/L, which means that there was a three to five times increased in healthy controls with a significance value of $p < 0.05$. In treatment group 1 (Ajwa date fruit extract 75mg/kgBW and 1 hour later given Meloxicam 30mg/kgBW) the results obtained ALT levels of 42.80 IU/L and AST 104.1 IU/L, treatment group 2 (Ajwa date extract 150mg/kgBW and 1 the next hour given Meloxicam 30mg/kgBW) the results obtained ALT levels of 34.90 IU/L and AST 93.26 IU/L, and treatment group 3 (Ajwa date fruit extract 300mg/kgBW and 1 hour later given Meloxicam 30mg/kgBW) ALT levels were 33.86 IU /L and AST 90.06 IU/L. From the analysis of ALT and AST levels above, it can be seen that the larger the dose of Ajwa date fruit extract in the treatment group, the lower the levels of ALT and AST significantly by 20-30% against healthy and negative controls.

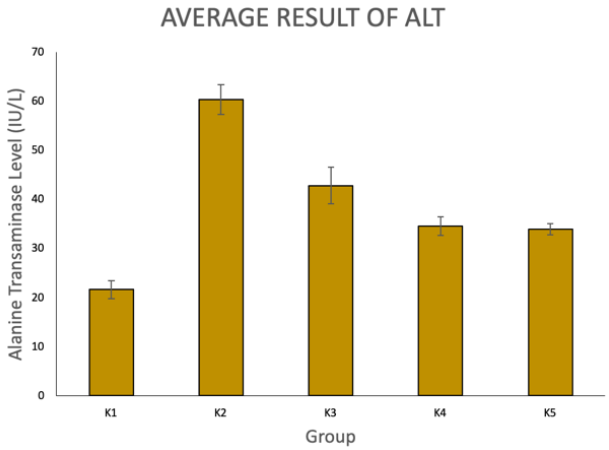

Figure 1. Diagram of the average ALT results in each group. The # symbol indicates $p<0.05$ (significant) for healthy controls, and the symbol * indicates $p<0.05$ (significant) towards healthy and negative controls

Liver histopathological preparations were made on the 15th day by taking samples by necropsy. The observations obtained are as follows:

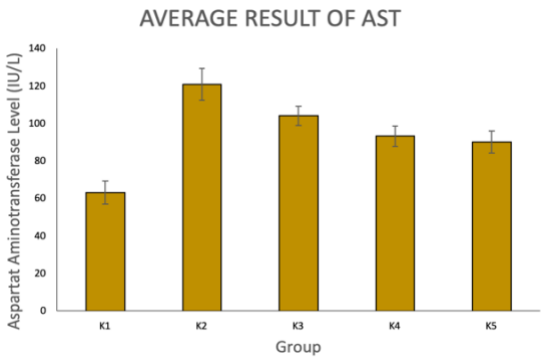

Figure2. Diagram of the average AST results in each group. The # symbol indicates p<0.05 (significant) for healthy controls, and the symbol * indicates p<0.05 (significant) towards healthy and negative controls

Table 1. Average score degree of liver damage in each group

Group	The average degree of liver damage
K1 (Healthy Control)	0
K2 (Negative Control)	2.8
K3 (Treatment Group 1)	2.2
K4 (Treatment Group 2)	1.4
K5 (Treatment Group 3)	1.2

Description: 0 = normal, no pathological changes; 1 = mild cell damage reaching 25%; 2= moderate cell damage reaching 50%; 3 = severe cell damage reaching 75%.

Comparison of the degree of liver damage in each group can be seen in the graph in Figure 3:

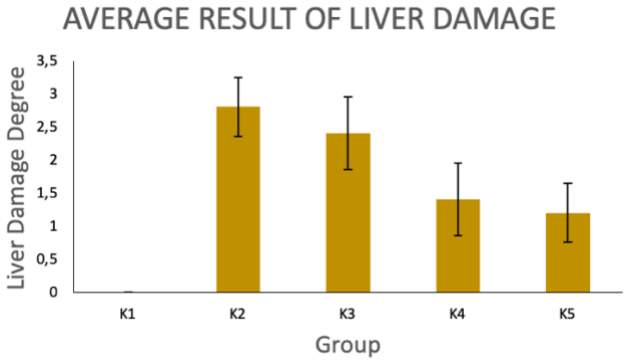

Figure 3. Diagram of the average liver damage in each treatment group. The symbol # indicates p<0.05 (significant) for healthy controls, the symbol * indicates p<0.05 (significant) for healthy and negative controls.

Statistical analysis showed that the degree of liver damage from healthy controls was 0.

Meanwhile, in negative controls there was a 3 times increased higher than healthy controls with an average degree of liver damage of 2.8. The negative control was given Meloxicam 30mg/kgBW without given Ajwa date fruit extract, so that statistical analysis showed a significant increase from the healthy control with $p<0.05$.

Then in the treatment groups 1, 2, and 3, there was a significant decrease of 15-20% where the average degree of liver damage obtained was 2.4 – 1.2. Treatment with 75, 150, and 300 mg/kgBW of Ajwa date fruit extract and the next 1 hour given Meloxicam 30mg/kgBW gave a significant decrease in healthy and negative controls. Statistical analysis can be concluded that the larger the dose of Ajwa date fruit extract in the treatment group, the lower the degree of liver damage significantly to healthy and negative controls with $p <0.05$.

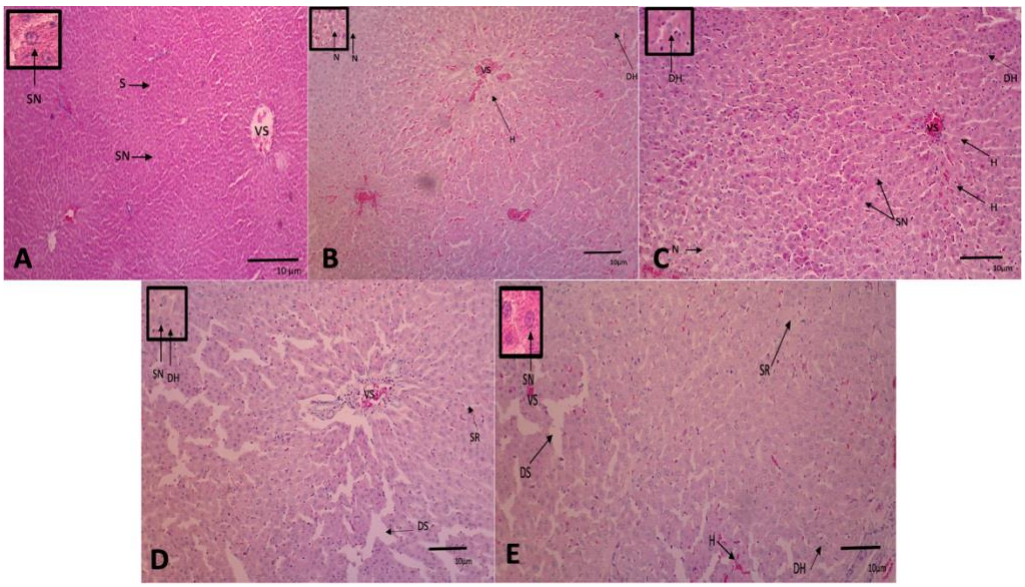

Figure 4. Histopathological images of the liver (HE, 10X). A: healthy control, B: negative control (Meloxicam 30mg/kgBW), C: treatment group 1 (Ajwa date fruit extract 75mg/kgBW and Meloxicam 30mg/kgBW), D: treatment group 2: (Ajwa date palm extract 150mg/kgBW) and Meloxicam 30mg/kgBW), E: treatment group 3 (Ajwa date extract 300mg/kgBW and Meloxicam 30mg/kgBW. Note: SN (Normal Cells), S (Sinusoids), VS (Central Veins), SR (Inflammatory Cells) , H (Hemorrhage), N (Necrosis), DH (Hydropic Degeneration), DS

Histopathological observations of the liver in the healthy control group showed that the hepatocytes were not damaged, and the sinusoids radiated centrifugally from the central vein. In the negative control (Meloxicam 30mg/kgBW) we can compare the differences in the appearance of liver tissue which has begun to experience severe or massive damage, with extensive inflammation of the cells characterized by the presence of hemorrhage or heavy bleeding. In the liver tissue also seen necrosis process which were characterized by the presence of hydropic degeneration. And the extent damage of the tissue reaches up to 75%.

Furthermore, the results of histopathological analysis of the liver in treatment groups 1, 2, and 3 showed a decrease in tissue damage by 25 - 50%. From the histopathological observations of the liver, it appears that the tissue were inflamed and moderately haemorrhagic, the tissue appears to have a little necrosis and hydropic degeneration. From the results of the liver histopathology analysis above, it can be concluded that the larger the dose of Ajwa date fruit extract in the treatment group accompanied by Meloxicam at a dose of 30 mg/kgBW can help reduce the degree of liver damage compared to the negative control group who were not given Ajwa date fruit extract at all.

Based on the results of the analysis of ALT, AST, and histopathological levels above, this can be related to research which states that Ajwa date fruit extract can help reduce the level of drug-induced increase in liver function (Hasna, 2015; Salem et al., 2018; Andrade et al., 2019) Other studies have also explained that the fruit extract Ajwa date palm has effectiveness as a hepatoprotector against CCl4 (carbon tetrachloride)-induced hepatotoxicity in adult rats, and restores antioxidant activity of liver enzymes by observing changes in liver function parameters that decrease or approach normal values. It is also known that Ajwa date fruit extract is rich in antioxidants and flavonoids which are thought to help reduce the cyclooxygenase process (COX-1 and COX-2) and act as anti-inflammatory in the incidence of degenerative diseases that trigger inflammation by reducing inflammatory mediators (Abdelaziz and Ali, 2014; Khalid et al., 2017; Khan et al., 2017; Isworo, 2020). Then the administration of Ajwa date extract (300 mg/kg/day for 14 days) significantly inhibited antioxidant depletion in albino rats when induced by lead acetate toxicity. This is due to the high phenolic content in Ajwa dates. The phytochemical properties of Phoenix dactylifera L. have been shown to provide beneficial biological and pharmacological effects including antioxidant activity and hepatoprotective effects (Ragab et al., 2013; Rahmani et al., 2014).

Hepatocyte damage due to continuous induction of Meloxicam for 14 days is thought to cause oxidative stress that induces membrane lipid peroxidation. Continuous administration of Meloxicam will form reactive oxygen species (ROS) which causes lipid peroxidation. Lipid peroxidation causes damage to cell membranes and causes cell structure to become abnormal and impair cell function (Kumar, 2005). Administration of Phoenix dactylifera L. extract was able to attenuate the pathological consequences of induction of PCM and CCl4 (Abdelaziz and Ali, 2014; Salem et al., 2018). Liver histopathological examination showed that Phoenix dactylifera L. fruit extract attenuated the incidence of liver lesions (including vacuolization and fibroblast proliferation). The hepatoprotective effect of Ajwa date fruit extract reduces elevated levels of serum liver enzymes and bilirubin. These findings indicate the ability of date palm extract to prevent liver cell necrosis (Abdelazis and Ali, 2014; Hall et al., 2016; Salem et al., 2018).

Conclusion

Based on the results of the research conducted, it can be concluded that Ajwa date palm extract (Phoenix Dactylifera L.) can provide a protective effect against liver damage in white rats (Rattus Norvegicus) due to Meloxicam, this can be seen by the decrease in liver function enzymes levels in this case. levels of Alananine Transaminase (ALT) and Aspartate Aminotransferase (AST), as well as changes in the histopathological picture of the liver which showed a decrease in the degree of damage to liver cell tissue.

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