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MACROSCOPICS AND MICROSCOPICS FEATURE OF WITAR RATS LIVER AFTER IMMERSION IN GRADUAL CONCENTRATION OF RANDU HONEY

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ABSTRACT

Background : Formaldeyde is main substance used for organ embalming and even for foods preservations. However, long and high exposure of formaldeyhde lead to various organs irritations. Honey is well-known for its natural preservative activity expected to be the soultion for the problem so there is safe alternative for organ preservations. **Aim :** Observation and examintaion the difference of macroscopic and microscopics feature of Wistar rats liver after immersion of formaldehyde and randu honey. **Methods :** Experimental with post-test only control group design using 24 male Wistar rats as sample, randomly divided into 4 groups consist of: Group C (preserved with 10% formaldehyde solutions), P1 (preserved with 10% randu honey solutions), P2 (preserved with 20% randu honey solutions) and P3 (preserved with 30% randu honey solutions). The sampels were preserved for 24 hours then macroscopic (smell,color and size) and microscopic study with Hematoxylin-Eosin (HE) stain using 400x observation performed. Manja Roenigk score were used to accessed the degree of live microsopic damage. **Results** : There are normal cells, mild damage, moderate damage and severe damage respectively in the group C: 36%,47%,17%,0% ; P1: 0%,27%,46%,27% ; P2: 0%,77%,17%,6% ; P3: 47%,33%,17%,3%. Kruskal-Wallis test showed significant results with p=0.000. With Mann-Whitney test, significant results were found between group C with P1 (0.000), C with P2 (0.006), P1 with P2 (0.000), P1 with P3 (0.000) and P2 with P3 (0.003). However, test result were not significant berween group C with P3 (0.684). **Conclusions** : There were differences in macroscopics and microscopic features between Wistar rats liver after immersion in formaldehyde and randu honey. The best features showed in samples preserved in 30% Randu Honey solutions.

***Keyword** : formaldehyde, honey, embalming, organ preservation*

INTODUCTION

Formaldehyde often used for preservations, even foods preservations. Formalydehyde used for preservations beacuse of its tendency to inhibit bacterial gorwth and other microorganisms.[1,2] However of formaldehyde has been associated with several organ irritations such as eyes and nose irritations.[3] In addition to formaldehyde side effects, oral inhalation of formaldehyde could lead to irrilations, formaldehyde could cause chromosomal damage if its taken orally.[2]

Randu honey is monofloral honey that could be easily found in Indonesia and honey has been a long-known natural preservative substance expected to be the solutions of the side efects from formaldehyde preservations. The preservations process in honey occurs naturally as honey contains of high glucose level and acidic pH which inhibit bacterial growth. Based on past study, randu honey has the best antibaterial effects as randu honey has the lowest pH and the smallest amount of bacteria left on the agar plate.[4,5]

This study use liver as the samples for liver has the distinct post-mortem presentations after 24-hours. The distinct prsentations in liver is called honey comb appearance which formed by enlarged gas bubble in the liver.[6]

METHODS

True experimental with post-test only control group design. Research conducted at Animal Laboratory of Diponegoro University. Inclusion criterias for the Wistar rats are male, age 90-120 days and healthy with no anatomical abnormality. While the exclusion criterias are letargic and sick rats.

Wistar rats obtained from the population at breeding center in Ungaran, Central Java. The honey were produced by Rahtu Madu a local certified randu honey supplier. Honey is stored tightly in glass container, dry, away form sunlight and at room

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temperature. 1 liter of honey used for this study. Several tests performed to verify the randu honey purity, such as honey visual selective test and quantitative test to obtain glucose level contained in the honey. Quantitative test is performed at PT. Sucoffindo Semarang with HPLC (High Performance Liquid Chromatography) methods.[7,8]

Samples selected using simple random sampling with 24 male Wistar rats as sample, randomly divided into 4 groups consist of: Group C (preserved with 10% formaldehyde solutions), P1 (preserved with 10% randu honey solutions), P2 (preserved with 20% randu honey solutions) and P3 (preserved with 30% randu honey solutions). Samples were adapted before this study performed.

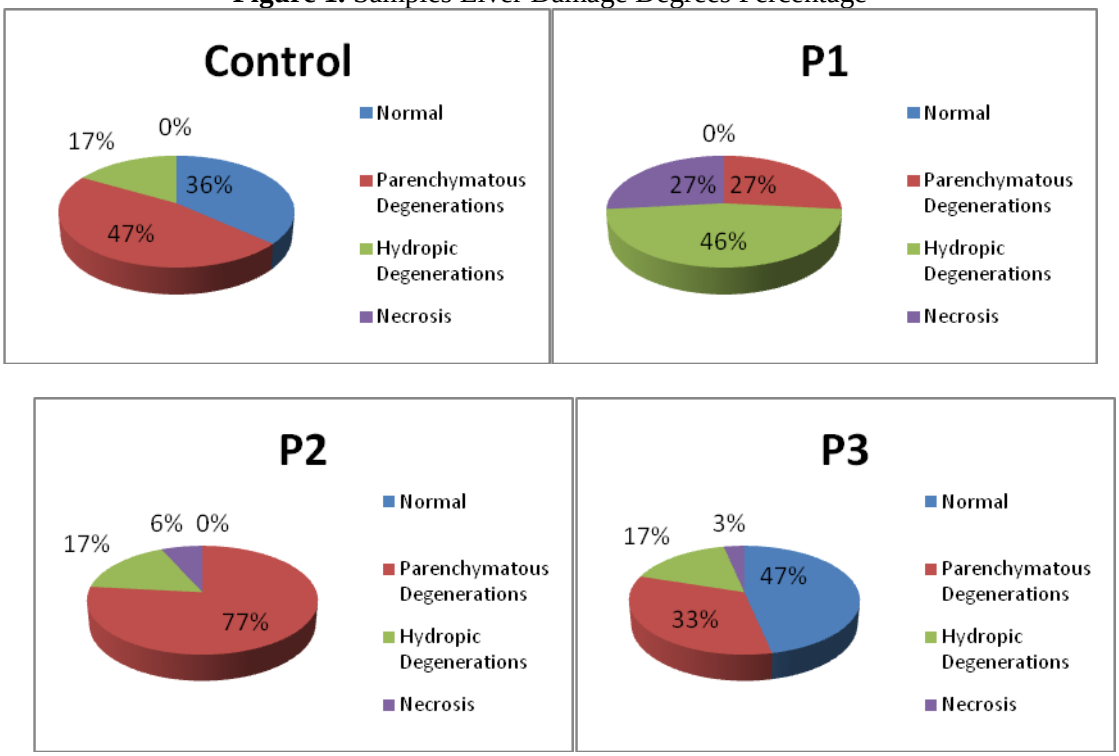
The independent variable in this study was randu honey concentartion and the dependent variable was macroscopic (smell,color and size) and microscopic of rats liver post-mortem prsentation. The sampels were preserved for 24 hours then macroscopic study perfomed immeadiately. Microscopic study preparations done right after using Hematoxylin-Eosin (HE) stain. Preparations were read with 400x magnifications in 5 different fields for each samples. Manja Roenigk score were used to accessed the degree of live microsopic damage. The data obtained was processed using computerized statistic application.

RESULTS

The effects of different fixative on the samples were identified based on macroscopic and microscopic examination. According to macroscopic examination, there was no significant difference among the groups. However, macroscopic examination were examined subjectively so microscopic examination was needed to obtain more reliable results.

The results for microscopic examination showed that there was significant difference between formaldehyde and honey according to *Kruskal-Wallis* test ($p<0.05$). *Kruskal-Wallis* test was choosen according to normality test results, showed that data acquired from this study were not distributed normally with $p=0.00$ ($p<0.05$).

Figure 1. Samples Liver Damage Degrees Percentage



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There was also a difference between C group with P1, C group with P2, P1 with P2, P1 with P2 and P2 with P3 ($p<0.05$) accroding to *Mann-Whitney* test. By contract, there was no significant difference between C group and P3 ($p>0.05$). However, looking at the frequency of normal cells between C group (36%) and P3 (47%). there was a slight difference where normal cells in P3 were slightly higher. *Mann-Whitney* done by considerations to know the best randu honey consentrations for preservations.

Figure 2. *Mann-Whitney* test results

Group Sample	C	P1	P2	P3
C	-	0.000*	0.006*	0.684*
P1	0.000*	-	0.000*	0.000*
P2	0.006*	0.000*	-	0.003*
P3	0.684*	0.000*	0.003*	-

**Mann-Whitney* test results are significant if $p<0.05$

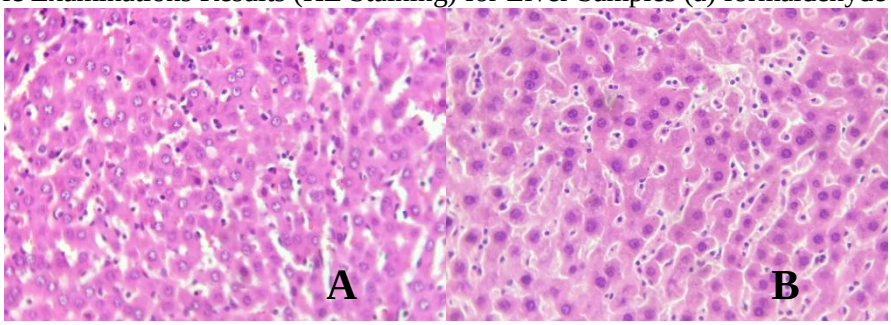
DISCUSSION

Formalydehyde is widely used as preservative subtances for embalming. But despite of its popularity as preservatives, formaldehyde also well-known for its disadvantages such as irriations and even classififed as carcinogenic.[1,9] Numerous attempts conduted to find subtitutes regarding to the disadvantages and carcinogenic properties of formaldehyde. Honey has been used for years for storing meat and mumification.[10] Honey has been shown to inhibit microorganisms growth leading to preservations. Antibacterial compounds of honey depend on its acidity, high glucose level and hydrogen peroxide (H_2O_2). Hydrogen peroxide (H_2O_2) generated after dilutions of honey with water and will reach its peak in 30% concentrations solutions.[5,11]

The quality of post-mortem tissues are much likely depends on its preservative substances. In this study, macroscopic and microscopic examinations performed to know its quality after preserved with honey compared to formaldeyhde. The results conducted from macroscopic examinations showed that there was not much differences between honey and formaldehyde. However, the macroscopic examinations affected with subjective issues, the reasons microscopic examinations was conducted.

Microscopic examinations performed showed the expected results. Tissue samples from liver preserved with 30% randu honey were better than samples preserved with formaldehyde in numbers of normal cells eventhough in statistic tests is not significant (47% and 36%). Although in both samples showed several damages of the rats liver, the results were aligned with past conducted study.[5,11,12]

Figure 3. Microscopic Examinations Results (HE Staining) for Liver Samples (a) formaldehyde (b) honey. 400X



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Degrees of liver damages such as parenchymatous degenerations and hydropic degenerations were still founded in both of the samples. Parenchymatous degenerations occured because of oxidation disruptions causing water accumulation and granulated cytoplasms. Hydropic degenerations is a result from ionic disruption leading to cell swelling, pale cytoplasms and vacuolizations. Results from C group and P3 are likely to be similar with most of the cells were normal and parenchymal degenerations showed which is a mild damage of the liver and aligned with past studies.[5,7,11,12]

In our study, microscopic examinations results showed that randu honey could be used as formaldehyde alternative regarding the similar and even better results in randu honey compared to formaldehyde. Organ preservatives study using other organs and types of honey should be undertaken for further ackowledgements.

CONCLUSION

There were differences in macroscopics and microscopic features between Wistar rats liver after immersion in formaldehyde and randu honey. The best features showed in samples preserved in 30% Randu Honey solutions.

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