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THE EFFECT OF MORINGA OLEIFERA L. AGAINST SERUM PROTEIN AND TISSUE IN PREGNANCY

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ABSTRACT

Moringa leaves are known as herbs that have many properties. The study aimed to analyzed moringa leaf extract against tissue histology, serum protein levels, fetal conditions at birth from distended mice with malnutrition. The research design was a pretest post *test* control *group design* using 28 distended mice with malnutrition, divided into 7 groups with 4 mice - Group. In the treatment group given moringa leaf extract with a dose of 720; 850 and 1000 mg/day during pregnancy 19-21 days. We found the condition of malnutrition causes hepar tissue histology to deteriorate due to increased hepar cell death which affects decreased protein synthesis, but does not affect renal tissue histology. The administration of moringa leaf extract does not have an effect on the distending of malnutrition conditions both the results of histology of hepar tissue and kidneys, serum protein levels and fetal conditions at birth, but in distending without malnutrition (negative control) provides the best results of tissue histology, serum protein levels and provides the highest fetal weight and length compared to other groups.

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Introduction

Pregnancy requires special nutrition because it greatly affects the development of the fetus it contains until the child is two years old or the first 1000 days of life, nutritional adequacy is very influential on the physical and cognitive development of the child. Indonesia Basic Health Research results in general in Indonesia in toddler with very short and short nutritional status of 29.9%, the proportion of women of childbearing age pregnant with upper arm circumference <23.5cm or risk of chronic energy deficiency in Indonesia by 17.3%% [1]. The results of research on the potential of moringa leaves that are useful to improve the condition of pregnancy have been done are: Iskandar showed that the administration of *Moringa oleifera* extract in pregnant women who suffer from anemia and have low body weight in Gowa, South Sulawesi, can increase hemoglobin levels by 58%, ferritin levels in serum by 50% and at the end of the study did not get pregnant women who have low weight [2]. Nadimin showed that giving *Moringa oleifera* extract to pregnant women who do not suffer from anemia in Makassar can increase hemoglobin levels although not as high as in the group of pregnant women who were given folic acid and iron tablets. [3] Suriati states that moringa nutritional content is very good for meeting nutritional needs for pregnant women and toddlers. In meeting the nutritional needs of pregnant women, pregnant women simply consume moringa leaf powder as much as 6 times a day at a dose of 50 grams per consumption [4]. Meanwhile, to meet the nutritional needs of toddlers, they need 25 grams of moringa leaf powder every time consumption as much as 3 times per day. Research using animals to model malnutrition that were pregnant has never been done so the author is interested in analyzing it in order to get improvements in good conditions in the mother and fetus. The study aimed to analyzed moringa leaf extract against tissue histology, serum protein levels, fetal conditions at birth from distended mice with malnutrition.

Materials and Methods

This research had an experimental design of study, with research design: *randomized pretest post test control group design*.

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Sample

Mus musculus (mice) strain Balb/C, female, adult, age 10 weeks, weight 25-30 grams, taken with random allocation from biochemistry laboratory of Airlangga University, Indonesia then made into a pregnant model animal with malnutrition.

Sample Size

Calculated by the formula (t-1) (n-1) $\geq$ 15, where t was the number of groups, n is the number of animals tried per group. So the minimum number of animals tried in each group is 4, and the total sample is at least 28.

Manufacture of Malnourished Model Animals

The malnourished model animals were made by way of a group of female mice fed low protein (a mixture of rice flour and amylum) for 30 days. To make a distended squeak, the squeak is selected in a known estrus period condition from the marks of the vulva. The estrus period of mice lasts 4-5 days with a period of estrus 12-14 hours. The female mice at the time of estrus grounded with the male mice with a ratio of 3: 1, then if the next day obtained *vaginal plaque* then begin to be calculated pregnancy day 0. Mice has a gestation period of 19-21 days with an average birth rate of 6 to 15 [5]. Surgery is done on the 21st day or during childbirth with a cervical dislocation without anaesthetic.

In this study used 28 squeaks, all mice were given a *pellet* starter and drank *ad libitum* during the adaptation period, then grouped into 7 treatment groups and each treatment group consisted of 4 mice. The manufacture of squeaking malnutrition models begins with low-protein feeding for 5 weeks, then continues with the process of cutting.

The group consists of:

1. Pre Test Group
2. Placebo Group: distended without malnutrition that is not given any treatment
3. Positive control group: distended group with malnutrition who were not given any treatment
4. Negative control group: the distended group without malnutrition is given moringa leaf extract
5. Moringa leaf extract treatment group (1): distended mice with malnutrition given moringa leaf extract equivalent to 720 mg / oral / day, using sonde
6. Moringa leaf extract treatment group (2): the group of distended mice with malnutrition given moringa leaf extract equivalent to 850 mg / oral / day, using sonde
7. Moringa leaf extract treatment group (3): the group of distended mice with malnutrition given moringa leaf extract equivalent to 1000 mg / oral / day, using sonde

During the treatment mice are fed and drank ad libitum. At the end of the observation, serum protein, fetal body count and weight and histology of heparous tissue and kidneys were performed.

Research Materials and Tools

Chemicals used in the study: moringa leaf extract, kit for examination of serum protein levels, 10% formalin, *Hematoxylin Eosin,* dry ice, will *pellet* starter and aquadest. The tools used in the study include: soxhlet, rotary *evaporator (BUCHI Rotavapor* R-114), water bath *electric (BUCHI Water Bath* B-480), Ohaus PA-214 analytical balance sheet,*) centrifuge*, micequer cage, animal scales, oral sonde, micropipet and tip, *conical tube, vortex,* microtome, test tube, *object glass*, paraffin, microscope, staining tub, *Cambridge Pad, spuit* 1 and 3 ml, a set of minor surgical instruments (for taking mice), and laboratory glass tools.

Making Moringa Leaf Extract

A total of 7.28 kg of wet moringa leaves are dried with sunlight so that 1.46 kg of dried moringa leaves are obtained by maceration and dried using *freeze dryer*

Giving Moringa Leaf Extract

Administration of moringa leaf extract equivalent to 720 mg / day; 850 mg / day and 1000 mg / day, using sonde in the morning, starting pregnancy days 0 to 21 / during childbirth [6, 7].

Analysis of Serum Protein Levels

The total protein in serum is measured by Biuret reagent and copper sulfate base. Spectral color absorption at λ 545 nm. Albumin levels are also measured by the same method, so globulin is calculated from the difference in levels between the total protein and albumin measured.

Analysis of Hepar and Kidney Tissue Histology

Liver tissue is taken, soaked in a 10% solution of formalin and then made preparations with paraffin. Thin layer the tissue slices with microtoms, followed by the staining of *Hematoxylin Eosin.* The count of the number of dead cells in the organ and expressed in % using a microscope.

Analysis of Fetal Body Count and Weight

The number of fetuses is calculated and the weight of each fetus is weighed by the balance sheet.

Results and Discussion

Animal Making Tries to Malnutrition

Squeak is carefully adjusted for 1 week to adjust to the environment. Mice were fed low protein made from a mixture of rice flour and amylum, and monitored with measurements of mice weight every week, linear regression results obtained an F value of 44,988 with a significance value of 0.000, indicating the process of making malnutrition is said to be successful. The graph is showed in **Figure 1**.

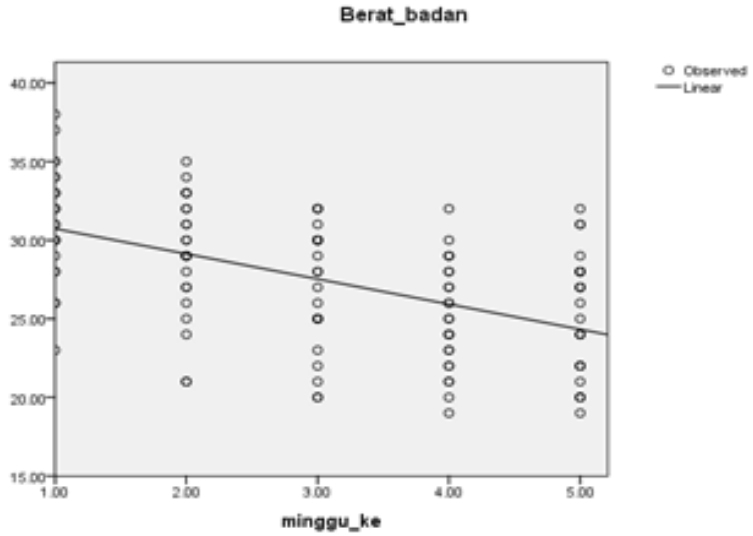


Figure 1. Weight loss chart squeaks when making animals model malnutrition for 5 weeks

After the manufacture of animals the model of malnutrition continued with the process of casting which was done by grounding 1 male squeak with 3 female mice. Mice have a gestation period of 19-21 days with an average birth rate of 6 to 15

Table 1. Number of distended mice and fetuses/groups, weight and body length of the newborn fetus each group

Group	Number of distended groups (tails)	Number of fetus born	weight of fetus (g)	Length of fetus (cm)
Placebo Group	4	7	2,97	2,93
Positive control group	2	5	1,87	2,82
Negative control group	4	7	4,01	3,89
Moringa leaf extract treatment group (1)	2	5	1,715	2,675
Moringa leaf extract treatment group (2):	2	5	2,015	2,845
Moringa leaf extract treatment group (3)	2	5	1,585	2,58

Table 1 obtained an overview of the condition of malnutrition makes squeak more difficult to distend than normal conditions and the average weight and body length of the fetus is best in the group of distended mice without malnutrition by giving moringa leaf extract (negative control).

Mice that are not distended in groups numbered 0-2 tails so that the data on the number of fetuses born is not too much. The effort that has been done is to replace males in cages; it turns out that these efforts have not received effective results. The difficulty of pregnancy in the mice group with malnutrition is thought to be due to hormonal participation. Estrogen and progesterone are produced in the ovaries to regulate the maturity of the egg by affecting the release of FSH and LH from the pituitary gland in the brain. The hormone FSH stimulates the development of follicles (will be eggs) that will later become mature eggs, while LH stimulates the release of mature eggs from the ovaries in the fertile period. These hormones require protein in their transport system so that the performance mechanism of hormones is also disrupted in the condition of malnutrition.

The best fetal weight and height is obtained in the group of fetuses born to the mother of mice without malnutrition by giving moringa extract means that the administration of moringa leaf extract improves the condition of distended squeaking with the birth of children who are weight and longer weight better than other groups. This is reinforced by the results of Sawitri's

research that there is a correlation of adolescent nutritional status with the regularity of the menstrual cycle that is indirectly related to hormonal [8].

If the mother is malnourished, then there can be abnormalities in the placenta in the form of placental vascular abnormalities or a decrease in the ability to transport by the placenta. Some parts of the placenta are actively involved in the transfer, synthesis and processing of nutrients under the influence of the fetus, mother and placental hormones. Women who are malnourished or women in low socioeconomic groups have less placental weight than women who come from higher social groups. The placenta of a malnourished woman has less DNA and protein. This suggests that many of the cells are reduced in the placenta of women suffering from malnutrition. In an analysis the surface of the villus is reduced so that the transfer area of the mother with the fetus is reduced as the baby's birth weight is related to the weight of the placenta.

If the mother is malnourished during pregnancy will cause many problems, both in the mother and fetus, as described below:

1. Undernutrition in pregnant women can cause risks and complications in mothers, among others: anemia, bleeding and susceptible to infection.
2. The influence of undernutrition on the labor process can result in difficult or long labor, premature labor and postpartum bleeding.
3. Malnutrition in pregnant women can affect the process of fetal growth and can cause abortus, stillbirth, neonatal death, congenital defects, anemia in infants, intra partum asphyxia, low birth weight (BBLR) to *stunting.*

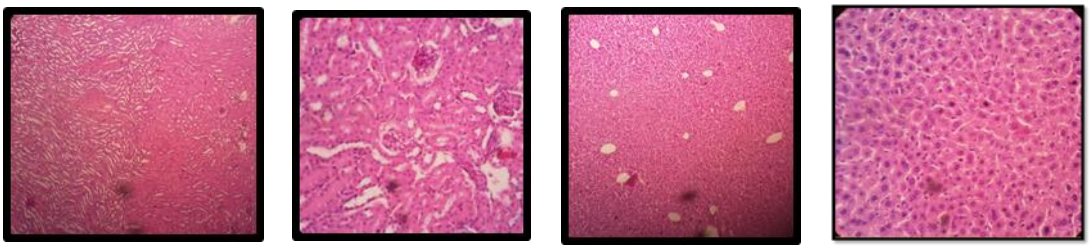
Histopathology of Tissues in the Condition of Distended Squeak

Hepar tissue histology examination aims to analyze damage in malnutrition conditions that will indirectly result in albumin production and total protein, while the renal tissue histology examination aims to see if the effects of malnutrition can cause kidney tissue damage. In addition, it can be studied whether the administration of moringa extract has toxicity related to the function of the liver and kidneys as excretory organs. This type of histological examination is the percentage of the number of dead cells in heparous and kidney tissue. Examination of tissue histology is carried out on all mice both successfully pregnant and non-pregnant in each group

Table 2. Percent of Cell death's Hepar and Kidney

Group	Average % of cells die in the kidneys	Average % of cells die in Hepar
Pre Test	0	0
Group placebo	0	0
Positive control group	0	52.5
Negative control group	0	0
Treatment group (1),	0	42,5
Treatment group (2)	0	42,5
Treatment group (3)	0	40

The histological condition of kidney tissue and hepar can be seen in the following image



Kidney(100x) Kidney (400x) Hepar(100x) Hepar (400x)

Figure 2. Microscopic kidney tissue and hepar with the condition do not get dead cells in the preparation.

100x 400x 100x 400x

(Hepar cells died 70%) (Hepar cells died 90%)

Figure 3. Microscopic kidney tissue and hepar with the condition there are dead cells in the preparation.

From **Table 2** and **Figure 2** obtained data that kidney tissue in mice in all groups, in good condition and obtained the percentage of cells die microscopically by 0% so it was concluded that malnutrition and the administration of moringa leaf extract is safe against kidney organs. From **Table 2**, **Figures 2 and 3** obtained data that the hepar tissue in mice is normal and that is damaged microscopically, especially in the group of distended mice with malnutrition.

Severe malnutrition conditions will interfere with metabolism and systems in the body, especially in the liver, so that it indirectly interferes with the process of protein synthesis in the body. The apoptosis pathway is triggered by the interaction between ligand death *(tumor necrosis factor* and *Fas ligand)* and receptor death *(tumor necrosis factor receptors* - 1 and *Fas)* characterized by cell shrinkage and fragmentation of nuclear chromatin. This interaction activates receptors that divide cellular proteins and eventually causes cells to die in cumulative doses [9]. Injuries to mitochondria in either mitochondrial structure, function, or DNA synthesis can interfere with lipid β-oxidation and oxidative energy production in hepatocytes. Severe damage to mitochondria eventually leads to liver failure and death. Malnutrition conditions, especially protein deficiency, cause hepatic cirrhosis, resulting in fatty liver and resulting in cell/tissue death.

Serum Protein Examination Results

Serum protein level examination is carried out on all mice (4 tails) in each group either in a state of being pregnant or not pregnant. The results of the analysis of total levels of proteins, albumin and globulin can be seen in the **Table 3**:

Table 3. Results of analysis stastitical of total levels of protein, albumin and globulin in serum

Group	Average Total Protein	Average Albumin	Average Globulin	Anova Test
Pre Test	7,2425	3,265	4,1275	
Placebo group	7,805	3,88	3,925	P Total Protein = 0,000
Positive control group	5,355	2,8725	2,4825	
Negative control group	8,4175	3,9925	4,425	P Albumin = 0,003
Treatment group (1),	6,7825	3,3325	3,3525	P Globulin = 0,000
Treatment group (2)	7,5325	3,705	3,8275	
Treatment group (3)	6,805	3,7425	3,0625	

In general, there are significant differences in the total levels of proteins, albumin and globulin from the malnourished and non-malnourished mice. Albumin maintains the oncotic pressure of colloidal plasma by 75-80% and constitutes 50% of all protein in the body. If plasma proteins, especially albumin, can no longer maintain colloidal osmotic pressure, there is an imbalance of hydrostatic pressure that will cause edema. Albumin serves as a transport of a wide variety of substations including bilirubin, fatty acids, metals, ions, hormones, and medications. Synthesis occurs only in liver cells with a production of about 15 g/day in healthy people, but the amount produced varies significantly in different types of physiological stress. The half-life of albumin is about 20 days, with a degradation rate of 4% per day [10], hypoalbuminemia can be caused by decreased albumin production, ineffective synthesis due to liver cell damage, lack of protein intakes, increased producers.

Hypoalbuminemia can be caused by decreased albumin production, ineffective synthesis due to liver cell damage, lack of protein intake, increased albumin expenditure due to other diseases, and acute and chronic inflammation. Amino acids are required in albumin synthesis, as a result of protein intake deficiency there is damage to the endoplasmic reticulum of cells that affects the synthesis of albumin in liver cells [11].

Conclusion

The provision of moringa leaf extract does not have a positive effect on (a) histology of kidney tissue and hepar parent mice where the parent hepar tissue of the mice is microscopically damaged by malnutrition. (b) The level of the parent protein of the mice because the amount of protein produced is highly dependent on the condition of the hepar tissue of the parent squeak suffers microscopic damage due to malnutrition. The administration of moringa leaf extract improves the condition of distended mice without malnutrition so that the fetus that is born has a heavy weight

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Conflict of interest: None

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Ethics statement: The use of these tried animals will get a certificate of ethical feasibility by the Health Research Ethics Commission of the Surabaya Ministry of Health confirmed this study following the Helsinki Declaration (Protocol №5, 26.05.2016).

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