Effect of Combination of Nano Herbal Andaliman (*Zanthoxylum acanthopodium* DC.) and Extra Virgin Olive Oil (EVOO) to Kidney Histology of Preeclampsia Rats

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**Abstract.** Preeclampsia is a pregnancy disorder that can cause interference in the kidneys. This study aims to determine the safety effects of the combination of Nano herbal Andaliman (*Zanthoxylum acanthopodium*) and Extra Virgin Olive Oil (EVOO) in preeclampsia. This study consisted of 5 groups ie K(-): Pregnant Rats, K(+):PE model Rats, P1: PE mode Rats + 0.45g EVOO/day/200gBW at 13th-19th days pregnancy, P2: PE mode Rats + Nano-Andaliman 100mg/200gBW at 13th-19th days pregnancy and P3: PE mode Rats + 0.45g EVOO/day/200gBW + Nano-Andaliman 100mg/200gBW at 13th-19th days pregnancy. This study showed no differences in kidney weight (p>0.05) but there were significant differences in Necrosis in kidneys (p<0.05). Combination of Nano herbal Andaliman (*Zanthoxylum acanthopodium*) and Extra Virgin Olive Oil (EVOO) in preeclampsia can effect in histology kidneys.

1. **Introduction**

Preeclampsia is a multisystem disorder in pregnancy, which complicates 3% -5% of pregnancies and is a major cause of maternal morbidity and mortality throughout the world. Recent data show that the contribution of preeclampsia is estimated to be around 5 times that of maternal-neonatal morbidity & mortality [1,2,3,4]. PE symptoms such as edema, headache, and severe cases (eclampsia), renal and hepatic dysfunction, and blood clotting disorders, Distress syndrome and *Intrauterine growth restriction* (IUGR) [5]. PE can cause interference with the kidneys. Kidney function was disrupted and damaged due to vasoconstriction and decreased perfusion in severe preeclampsia. Increased creatinine and proteinuria levels indicate glomerular function disorders, and most cases of kidney failure are caused by acute tubular necrosis [6]. Natural antioxidants are known to have beneficial effects [7,8,9,10] on hepatitis or liver disorders induced by antitubercular agents [11].

Andaliman is a typical plant of the Batakese ethnic, North Sumatra, Indonesia. Andaliman extract contains quite a lot of chemicals such as alkaloids and steroids and the researcher have shown that the content of terpenoids has antioxidant and antimicrobial activity (anti fungus, anti-bacterial), the repellent and kill the insects[12,13,14]. Coumarin and alkaloids are the typical ingredients of the *Zanthoxylum* family [15,16]. The fruit of the genus Zanthoxylum is usually used in the digestive system, heals as thma and bronchitis, relieves pain, to treat heart disease, mouth, tooth, throat, dyspepsia and diarrhea diseases. Zanthoxylum can be used as an antinociceptive, anti-inflammatory, antiplasmodial, cytotoxic, antiproliferative, anthelmintic, antiviral, anticonvulsant, antifungal, analgesic, antibiotic and hepatoprotective agent [17-22].
Nano technology in herbal medicine [23,24] can be targeted at an organ such as the brain, lungs, kidneys and digestive tract with selectivity and high effectiveness and safety, besides that the release of active compounds can be controlled so as to minimize the effect [16]. Extra virgin olive oil (EVOO) from Olives fruits contain antioxidants ie Vitamin E, hydroxytyrosol and tyrosols [10]. The combination of Nano-Andaliman and Extra Virgin Olive Oil (EVOO) is expected to reduce damage to the kidneys of preeclampsia.

2. Materials And Methods
This research is an experimental research, conducted at the Biology laboratory at the University of North Sumatra, Indonesia. The material used are Andaliman fruits obtained from Dairi in North Sumatera. This research use the Completely Randomized Design (CRD) using 25 pregnant Rats with an average weight of 180-200g. Rats are kept in Animal Cages Biological Laboratory, Faculty Mathematics and Natural Science, Universitas Sumatera Utara.

Andaliman Fruits is made into a nanoscale using High Energy Milling (HEM) [18]. The treatment consisted of 5 groups consisting of 5 pregnancy rats ie K(-): pregnant rats without any treatment, K(+): PE model Rats, P1: PE model Rats + 0,45g EVOO/day/200gBW at 13rd-19th days pregnancy, P2: PE model Rats + Nano-Andaliman 100mg/200gBW at 13rd-19th days pregnancy and P3: PE model Rats + 0,45 g EVOO/day/ 200gBW + Nano-Andaliman 100mg /200gBW + at 13rd-19th days pregnancy. Pregnant rats were dissected on the 20th day of pregnancy and Preparation of histologist by paraffin method and staining with Hematoxylin Eosin (HE) to observe of damage of kidney cells due to the administration of Combination Nano-Andaliman and EVOO. Dose of Nano-Andaliman was from toxicity test with Thompson Weil's formula [26,27]. The data processed with SPSS 22 program with Kruskal Wallis test.

3. Results and Discussion
3.1. Weight of Kidneys
Based on statistical data on renal weight in pregnant rats, there was no significant difference (P> 0.05) in each treatment in the right or left kidney. The highest mean weight of the kidneys was in the K+ and P3 groups, and the lowest in P2 group (Fig.6). Based on these data the EVOO [2,3,28], Nano-Andaliman and combination of both did not affect the renal weight of pregnant rats.

![Figure 1. Weight of Kidneys. K(-): Control; K(+): Preeclampsia; P1: EVOO, P2: Nano-Andaliman; P3: Combination of EVOO and Nano-Andaliman ( X ± SD).](image-url)
3.2. Necrosis and Narrowed tubulus

Based on statistical data, renal necrosis in pregnant rats showed a significant difference (P< 0.05) in group K- with K + and P1, K + group with P1, P2 and P3 (Fig.2A). The highest average value of necrosis was in the K + group and the lowest value of necrosis was in the control group and P3. Based on these data it is known that EVOO and both combinations are more effective in reducing renal necrosis in PE rats compared to Nano-andaliman only. Metabolic products in the form of toxic compounds released by the kidneys can cause kidney dysfunction, but the toxicity also depends on the dose, and decreases in body fluid volume which can predispose to toxicity [29-30]. Based on the percentage of renal tubular narrowing, the highest percentage was K + and P2, and the lowest was K-. (Fig.2B). Based on research data, it is known that Nano-Andaliman cannot improve tubular narrowing in the kidneys. This was suspected because of the Alkaloid content in Andaliman. Alkaloids contain at least one atom, and most nitrogen atoms are part of heterocyclic rings, and have certain physiological activeness [31]. EVOO has vitamin E (Tocopherol) which is anti apoptotic [32]. Cell apoptosis is part of cell death other than necrosis [33-35,24]. Olive converted to Extra virgin olive oil (EVOO) has analgesic, anti-inflammatory, and anticancer properties [36]. Kidney disease have a high risk of pregnancy disorder, although until now no reports of maternal death, the birth of premature infants or neonatal death in PE [37].

![Figure 2](image_url)

**Figure 2.** A. Necrosis of kidneys, B. Percentage of Narrowing tubulus. K(-): Control; K(+): Preeclampsia; P1: EVOO, P2: Nano-Andaliman; P3: Combination of EVOO and Nano-Andaliman (X ± SD).

3.3. Kidney Histology

Based on histological observations of the kidneys, proximal tubular damage is present in K +, but the administration of EVOO and Nano-andaliman slightly improves cell damage. Tubular narrowing is clearly seen in K + and P1 (Fig.3). From the histological picture it can be seen that Nano-Andaliman can cause narrowed tubules in pregnant rats (Fig.3). Kidney damage in PE can cause proximal tubular epithelial cells to swell with the granular cytoplasm. Swelling that occurs causes the proximal tubular lumen to narrow to close (Fig.3). Toxic substances that are thought to exist in Andaliman will accumulate in the kidneys, and cause damage to the kidneys with high blood flow to the kidneys,
cause various drugs, and chemicals in the systemic circulation [38]. The proximal tubular epithelial cells are sensitive to anoxia, and are easily destroyed by poisoning, due to contact with substances that are excreted through the kidneys, which can cause the pale cortex, enlarged kidney and edema, pyramidal congestion, vacuolated cytoplasm of epithelial cells in proximal tubules [39-42]. Based on this, it is known that the narrowing of tubules in PE Pregnant rats cannot be corrected significantly with Nano-Andaliman, but can be done with a combination of EVOO and Andaliman.

![Figure 3. Histology of kidneys. K(-): Control; K(+): Preeclampsia; P1: EVOO, P2: Nano-Andaliman; P3: Combination of EVOO and Nano-Andaliman. a) Necrosis b) Normal Tubulus c) Narrowing tubulus.]

4. Conclusion
Combination of Nano herbal Andaliman (Zanthoxylum acaanthopodium) and Extra Virgin Olive Oil (EVOO) can be effect in safety in kidney but the percentage of tubular narrowing is found in the Nano-Andaliman group. So it is recommended that consumption of Andaliman combined with EVOO in pregnant.

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