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RESEARCH ARTICLE

The effect of ethanol extract of *Andaliman* (*Zanthoxylum acanthopodium* DC.) on kidney damage in tartrazine-induced rats

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ABSTRACT

Tartrazine is a yellow synthetic dye with aromatic and chromo groups. It is widely used in food colouring products and cosmetics, and prolonged consumption can cause kidney damage. *Andaliman (Zanthoxylum acanthopodium* DC.) is a North Sumatra-native spice plant which reported as antioxidant to repair kidney damage. This study was conducted to determine the effect of ethanol extract of *andaliman* fruit on the morphology, ureum levels, histology, and glomerular diameter of tartrazine-induced rats. This study employed a completely randomised design (CRD). The ethanol extract of *andaliman* fruit was dissolved in CMC-Na 1%. Twenty-five rats were divided into 5 groups: negative control (KN), positive control (tartrazine indcution/PN), and treated groups (P1, P2, and P3 induced tartazine and treated with *andaliman* extract at dose 150 mg/kg BW, 300 mg/kg BW, and 450 mg/kg BW, respectively). After 30 days treatment, kidney damage was observed based on colour and surface of kidneys, kidney index, ureum level, kidney damage score, and glomerular diameter. There are no significant different in the kidney index among groups ($p > 0.05$). Tartrazin at a dose of 15 mg/kg BW was able to induce the kidney damage as indicated by the increase ureum levels, kidney damage score, and the decrease of glomerular diameter in the negative control. The treatment of *andaliman* fruit ethanol extract was able to repair the tartazine-induced kidney damage as indicated by the decrease of ureum level and kidney damage score and incread the glomerular diameter.

1. Introduction

Tartrazine is one of the yellow synthetic dyes with an aromatic and chromophore group that is widely used in food colouring, cosmetics, and other products (Mustika *et al*, 2015). The use of tartrazine in Indonesia is governed by the Minister of Health's regulation on food additives, number 722/Menkes/Per/IX/88. The acceptable daily intake (ADI) for tartrazine in food is 0–10 mg/kg BW (BPOM, 2019). Large-scale and long-term use of tartrazine as a synthetic dye will cause damage to body organs, particularly the kidneys (Khayyat *et al.*, 2017).

The kidneys are bean-shaped excretory organs that are approximately 11 cm in length and serve as filters, reabsorbers, and secretors of urine and excretion (Ungu, 2020). The decline in kidney function, as measured by ureum levels, is an indicator of kidney damage caused by physiological factors. Ureum is the product of the body's metabolic breakdown of proteins (Hasanah *et al.*, 2020). Damage to the glomerulus and tubules diminishes the glomerular filtration rate, resulting in impaired protein filtration in the kidneys (Surya *et al.*, 2018). According to Shafira *et al.*, (2019) antioxidant in plants can treat kidney damage caused by toxic substance exposure. *Andaliman* is one of the

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plants that reported to contain antioxidants.

Andaliman (*Zanthoxylum acanthopodium* DC.) is a spice plant native to North Sumatra usually grows wild in the Tapanuli area and is used as a spice ingredient in ethnic cuisine of Batak Angkola and Mandailing (Sitanggang *et al.*, 2019). A *Andaliman* fruit is commonly used as the primary seasoning in Batak tribal cuisine, such as carp stew, naninura, and saksang (Sinaga & Prasetyo, 2019). Secondary metabolite compounds such as alkaloids, flavonoids, phenolics, saponins, and tannins have been identified in *andaliman* fruit extract through qualitative phytochemical analysis (Syarif *et al.*, 2015).

2. Materials and Methods

2.1. Study Design

This study was conducted in the biology laboratory of the Faculty of Science and Technology at the University of North Sumatra in Medan using a randomised design. Animal Research Ethics Committee of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara Number 0662/KEPH-FMIPA/2022 has granted ethical approval.

2.2. Extraction of Andaliman fruits

The *Andaliman* fruits (*Zanthoxylum acanthopodium* DC.*)* was collected from Raya Huluan village, Simalungun Regency, North Sumatra, Indonesia. Ten kg of fresh andaliman fruit were washed and separated from the branches before being sun-dried for 3 days. The dried samples were pulverised in a blender, then sieved to produce a powder. A total of 2 kg of *Andaliman* fruit powder was extracted by macerating for 20 h using 96% ethanol at ratio of 1: 10. A rotary evaporator is then used to concentrate the filtrate that has been filtered.

2.3. Animal Study Animal Model Preparation

Twenty-five male Wistar rats (*Rattus norvegicus* L.) weighing between 150 and 180 g were acclimatised for 7 days prior to receiving any treatment. The ethanol extract of *andaliman* fruit was dissolved in CMC-Na 1%, while the tartazine was induced orally at dose 15 mg/kg BW. Twenty-five rats were divided into 5 groups: negative control (KN), positive control induced tartrazine 15 mg/kg BW (KP) and treated groups (P1, P2, and P3 treated with *andaliman* extract at dose 150 mg/kg BW, 300 mg/kg BW, and 450 mg/kg BW, respectively).

2.4. Kidney damage observation

After 30 days treatment, the kidney damage was observed based on colour and surface of kidneys, kidney index values, ureum levels, scoring of kidney damage, and glomerular diameter. The kidney morphology was oberved based on the colour and surface of the kidneys. Kidney organ index was calculated by divided the kidney weight to the body weight of rats multiplied by 100%.

The level of ureum was measured form the blood serum collected from the orbital sinuses of rat eye. Normal ureum blood levels in rat is 15.0-21.0 mg/dl (Laksmi *et al.*, 2014). Observations of kidney histology was done by scoring of kidney damage according to the Roenigk Manja scoring model, namely: normal (score 1), bleeding (hemorrhagic) or parenchymal degeneration (score 2), fatty degeneration or hydropic degeneration (score 3) and necrosis (score 4). Glomerular diameter measurement utilising Image J software and five

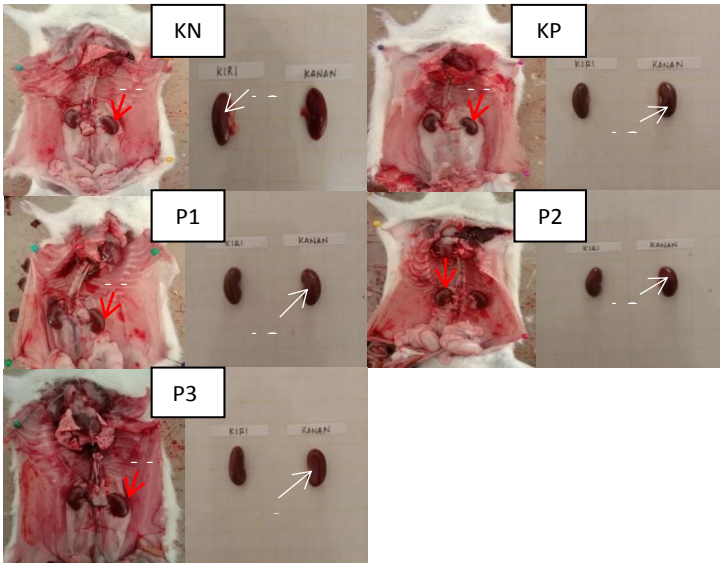

Figure 1. Renal morphology of rats of negative control (KN), positive control induced tartrazine 15 mg/kg BW (KP) and treated groups (P1, P2, and P3 treated with *andaliman* extract at dose 150 mg/kg BW, 300 mg/kg BW, and 450 mg/kg BW, respectively)

viewing fields.

2.5. Data Analysis

Data were analysis using the SPSS for windows.

3. Results

Observations of kidneys morphology revealed that administration of andaliman fruit ethanol extract and tartrazine for 30 days had no effect on the colour and surface of rat kidneys. In the KN group as the negative control the kidney morphology showed brownish-red kidneys with slick surfaces, while at the KP as the positive control group looked a brownish-red kidney hue and a slippery kidney surface. In thetreated group of *andaliman* fruit ethanol extract (P1, P2, P3) showed brownish-red, white rat kidneys, and slippery surfaces (Figure 1).

Table 1 Shows that the administration of tartrazin

Table 1. The kidney index, ureum level, kidney damage score, and glomerular diameter among groups

Variables	Treatment groups					*p* values
	KN	KP	P1	P2	P3	
Kidney Index (%)	0.74 ± 0.04	0.77 ± 0.05	0.78 ± 0.06	0.72 ± 0.03	0.77 ± 0.06	0.328
Ureum level (mg/dl)	19.00 ± 1.58^{a}	38.00 ± 1.58^{e}	31.20 ± 1.92^{d}	28,40 ± 2.07^{c}	22.00 ± 1.58^{b}	0.000*
Kidney damage score	25.60 ± 2.07^{a}	42.80 ± 1.30^{e}	38.40 ± 2.88^{d}	35.80 ± 1.09^{c}	33.00 ± 1.87^{b}	0.000*
Glomerular diameter (µm)	85.24± 3.47^{c}	65.87 ± 5.10^{a}	72.02 ± 9.65^{ab}	75.42 ± 5.57^{b}	76.20 ± 7.19^{b}	0.003*

Notes: * Indicates that there is a significant difference (p<0.05) based on the one-way ANOVA test. The different letters in the same row show there is a significant difference between groups.

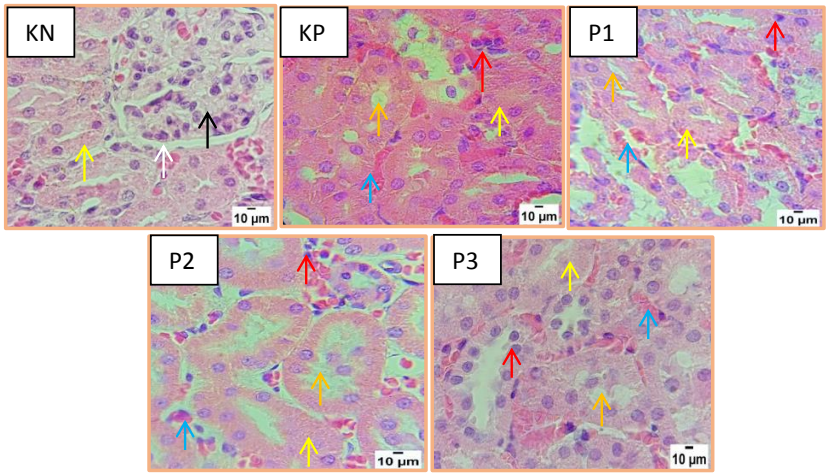

Figure 2. Histopathology of the kidney rats of negative control (KN), positive control induced tartrazine 15 mg/kg BW (KP) and treated groups (P1, P2, and P3 treated with *andaliman* extract at dose 150 mg/kg BW, 300 mg/kg BW, and 450 mg/kg BW, respectively). Black arrow shows glomerulus; white arrow shows capsule bowman; yellow arrow shows distal tubule and hydropic degeneration; green arrow shows proximal tubule; blue arrow shows bleeding (hemorrhage); orange arrow shows fatty degeneration; (red arrow) shows necrosis. H&E staining (100 x magnification).

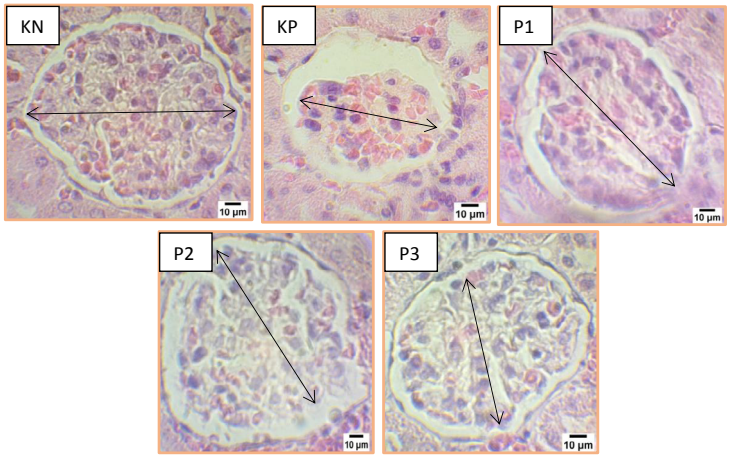

Figure 3. Glomerular diameter measurement using image J application of the kidney rats of negative control (KN), positive control induced tartrazine 15 mg/kg BW (KP) and treated groups (P1, P2, and P3 treated with *andaliman* extract at dose 150 mg/kg BW, 300 mg/kg BW, and 450 mg/kg BW, respectively). H&E staining (100 x magnification).

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and ethanol extract of *andaliman* fruit for 30 days did not affect the kidney index value ($p > 0.05$). The ureum level, kidney damage score, and glomerular diameter were different significantly among groups ($p<0.05$). Administration of 150 mg/kg BW, 300 mg/kg BW dan 450 mg/kg BW at P1, P2, and P3 respectively showed a significant reduction of ureun level and kidney damage score compared to tartazine-induced rats (KN). The lidney glomerular diameter of *andaliman* treated groups (P1, P2, P3) were significantly increased compared to the to tartazine-induced rats (KN). The histopatholgy of kidney and its glomerular are shown in Figure 2 and Figure 3.

4. Discussion

In this study, tartrazin at a dose of 15 mg/kg BW was able to induce the kidney damage as indicated by the increase ureum levels, kidney damage score, and the decrease of glomerular diameter in the negative control group. We also calculated the organ index as one of the parameters the adverse effects of a toxic compound in a specific organ (Hasibuan *et al*., 2015), although there are no significant different in the kidney index among groups ($p > 0.05$).

Tartrazin, a synthetic dye triggered the formation of reactive oxygen species (ROS) which induced the kidney damage. Oxidative stress caused glomerular damage thus induced re-accumulation of ureum in the blood (Yulyani *et al*., 2017). A chain reaction between free radicals and lipids damaged the cell membrane's structure (lipid peroxide) (Visweswaran and Krishnamoorthy, 2012), as indicated by the morfology and histopathological features of the kidneys. The tratazine-induced rats showed a glomerular shrinkage or known as glomerular atrophy which was characterised by the increase of glomerular diameter. According to Purba *et al*. (2021), the production ROS induced by tartazine caude the damage of endothelial cells, podocytes, and mesangial cells in kidney glomerular.

Functionally, the kidneys are heavily drained by blood and serve as a blood filter through the process of urine formation; therefore, the colour of the kidneys is a result of the kidneys' own functions (Guyton & Hall, 2007). This result is consistent with the research by Anggraini (2008). The normal rat kidney has brownish-red characteristics. The slick surface of the kidneys, unaccompanied by changes such as the spotted surface of the kidneys, is one indication that the kidneys are in a macroscopically healthy state. This is consistent with the findings of Togatorop *et al*. (2016), according to which a macrophotograph of the kidney of a white rat reveals that its surface is slippery. Based on the findings, the kidneys' morphology is brownish-red, and their surface is slippery.

The treatment of *andaliman* fruit ethanol extract was able to repair the kidney damage as indicated by the decrease of ureum level ureum levels and kidney damage score. Furtheremore, the glomerular diameter as increased significantly compared to the tartazine-induced rats. According to the results of the phytochemical analysis, *andaliman* fruit ethanol extract contains secondary metabolite compounds, including flavonoids, terpenoids, steroids, and tannins. Flavonoids are one of the polyphenol compounds with antioxidant properties. The hydroxyl ketones in flavonoids act as metal chelates, which are catalysts in the lipid peroxidation process (Adawiah *et al*., 2015). Antioxidant in *andaliman* fruit ethanol extract donates an electron to free radicals thus neutralised the lipid peroxidation process. In addition to being a group of polyphenol compounds, flavonoids consist of two aromatic groups A and one aromatic group B connected by a carbon bridge. By inhibiting, degrading, and regulating or protecting the formation of ROS, flavonoids are able to reduce the formation of free radicals (Alfaridz *et al*., 2016).

5. Conclusion

The treatment of *andaliman* fruit ethanol extract was able to repair the tartazine-induced kidney damage as indicated by the decrease of ureum level and kidney damage score and incread the glomerular diameter.

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