The effect of Angkak and a toxic dose of erythrosine on histopathology of mice (Mus musculus) kidney

by A W Jamaluddin

FILE	7._INDAH_ANGKAK_HISTOPATHOLOGICAL_MICE_KIDNEY.DOCX (1.35M)		
TIME SUBMITTED	14-DEC-2020 03:45PM (UTC+0700)	WORD COUNT	2619
SUBMISSION ID	1474523972	CHARACTER COUNT	13905

The effect of Angkak and a toxic dose of erythrosine on histopathology of mice (*Mus musculus*) kidney

R T Esa, D K Sari and A W Jamaluddin

Study Program of Veterinary Medicine, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia

E-mail: reskitenriesa_97@yahoo.com

Abstract. Angkak was a traditional fermented natural product originating from China. Some advantages of Angkak as food coloring were a more consistent and stable color, the ability of pigments to dissolve in water, the colors could be mixed with other pigments and were safe for consumption. Erythrosine was one of the synthetic dyes used as a food coloring. The compound was known to cause thyroid tumors, nerve disorders, and damage to chromosomes. Erythrosine was usually used in animal products as a dye in the sausage wrap. This study aimed to determine the effect of Angkak and Erythrosine on the histopathological features of mice kidneys. This study used 15 male mice, which were divided into five groups. The control group was given NaCMC, the treatment group 1 (KP1) was given Angkak at a dose of 0.0523 g, the treatment group 2 (KP2) was given Angkak at a dose of 0.523 g, the treatment group 3 (KP3) was given Angkak at a dose of 5.230 g, and treatment group 4 (KP4) was given erythrosine at a dose of 13.227 g for 30 days. All treatments were given peroral. On the day-31, all mice were necropsied, and the kidneys were taken for histopathological preparations with Hematoxylin-Eosin (HE) staining. The histopathological features were analyzed descriptively. The results showed the narrowing of the lumen, congestion, hemorrhage, necrosis, and infiltration of inflammatory cells were most commonly found in treatment KP4 given a toxic dose of erythrosine. In KP1, KP2, and KP3 given Angkak, there was no severe damage compared to KP4.

1. Introduction

Food additives are ingredients that are added intentionally to food in small amounts to improve appearance, taste, texture, flavor, and extend shelf life [1]. Food coloring is one of the food additives which is often used in various types of processed foods and drinks. Color is one of the essential properties of food, as well as good nutritional value, taste, and texture. The addition of dyes in foods and drinks has a considerable influence on the tastes and attractiveness of consumers in choosing a product [2].

Some synthetic food additives have been banned because of their toxicity. Also, the use of legal synthetic food coloring is still controversial because some studies show that synthetic food coloring harms human health, for example, brilliant blue, tartrazine, citrus red, carmine, amaranth, etc. [3]. The dangerous coloring agent commonly added by manufacturers was erythrosine. Erythrosine was occasionally used in animal products as a sausage wrapping color. Erythrosine dyes were prohibited for use in food because they are

known to be carcinogenic. Erythrosine caused thyroid tumors, nerve disorders, and damage to chromosomes [4]. Erythrosine also inhibited enzyme production in the kidneys. Research conducted in 2015 revealed the use of erythrosine in street food around an elementary school [5].

Unlike erythrosine, Angkak is a natural food coloring. Angkak was produced from rice fermentation with *Monascus purpureus* molds. People referred Angkak to red yeast rice as Chine rice because the product was red, made from rice, and historically originated in China. In the art of traditional Chinese medicine, red yeast rice was used for the treatment of indigestion, muscle wounds, dysentery, and cholesterol-lowering [6]. Angkak was also used to ease the work of the stomach and strengthen the function of the spleen, an organ that broke down red blood cells and filter out foreign compounds [6]. This research was conducted to compare the effects of natural and synthetic food colorings given to experimental animals through renal histopathological features.

Materials and methods

The sample used in this stud was 20 male mice, the age of 2-3 months with a bodyweight of approximately 20 to 25 grams. Samples were divided into five groups, the negative control group by administering NaCMC, treatment group 1 (KP1) was given Angkak 5 mg/kg BW of human dose, converted to a mouse dose of 0.0523 g which was suspended in 50 ml NaCMC or given in 0.313 mg/0.3 mL, treatment group 2 (KP2) was given Angkak 50 mg/kg BW of human dose, converted to a mouse dose of 0.523 g which was suspended in 50 mL NaCMC or given in 3.13 mg/0.3 mL, treatment group 3 (KP3) was given Angkak 500 mg/kg BW of human dose, converted to a mouse dose of 5.230 g which was suspended in 50 mL NaCMC or given in 31.38 mg/0.3 mL. Treatment group 4 (KP4) was given erythrosine at a dose of 1264 mg/kg BW of human dose, converted to a mouse dose of 13.22 g, which was suspended in 50 mL NaCMC or given in 79.36 mg/0.3 mL.

The number of experimental animals in this study was calculated using the Federer formula (n-1) (t-1)> 15, where n = repetition size and t = number of groups. In this study, there was one positive control group and three treatment groups. The repetition size for each group was at least four male mice; a total of 20 mice animals were used, which consisted of 16 male mice for treatment groups and four male mice for the negative control group. Before treatment, experimental animals were acclimatized for seven days according to the procedure operational standard of the Animal Education Clinic, Hasanuddin University. During the acclimation process, the humidity and cleanliness of the cage were maintained to keep all animals healthy. Husk was used as litter and replaced once every two days. Each mouse was given 5 grams of food a day and drinking water provided ad libitum. The treatment was given for 30 days, and all clinical symptoms were observed. The animals were euthanasia with aerosol alcohol ether, before necropsy. The kidneys were taken for histological preparations with hematoxylin and eosin (HE) staining.

3. Results and discussion

Gross examination in the control group, KP1, KP2, and KP3, did not undergo macroscopic changes. Kidneys looked normal with a brownish-red color, shaped like a kidney bean, and had a chewy consistency [7]. Significant changes were seen in KP4 with the treatment of erythrosine, which showed changes in color to blackish-red, slightly flattened at the bottom, and the tip became pointed. The blackish-red color in the kidney showed bleeding, while changes in kidney shape were possibly due to the narrowing of the tubules.

Table 1. Macroscopy observation of the kidney

Group	Structure		
	Color	Shape	Consistency
KK	Brownish-red	Kidney-bean	Chewy
KP1	Brownish-red	Kidney-bean	Chewy
KP2	Brownish-red	Kidney-bean	Chewy
KP3	Brownish-red	Kidney-bean	Chewy
KP4	Blackish-red	Slightly flattened at the bottom and pointed tip	Slightly harden

Observation of kidney histopathology in the control group (KK) did not show severe damage. Hemorrhage was found in some parts of the intratubular. Microscopically, a normal feature was seen both in the cortex and renal medulla (figure 1). The KK group showed a small amount of hemorrhage that occurred in the intratubular region. Hemorrhage was a condition characterized by the release of blood in the vascular due to damage to the vascular wall [8]. The hemorrhage was not due to the NaCMC, but could be caused by trauma, infection, vitamin C deficiency, and toxic exposure [9].

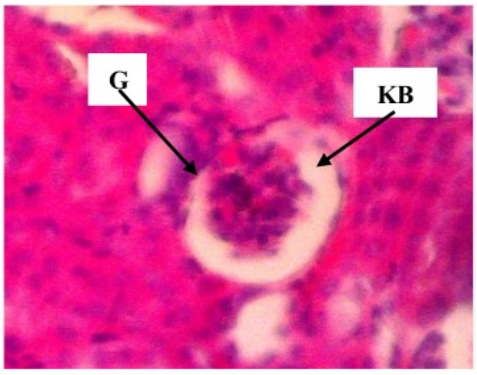

Figure 1. Microscopic features of KK1 (HE staining, 40×10). Glomerulus **(G)**, Bowman's capsule **(KB)**.

The KP1 group showed mild damage to the kidneys. Damages that occurred were hemorrhage, necrosis, and accumulation of inflammatory cells (figure 2). As explained before on KK, hemorrhage was not only caused by the treatment given. Still, it can also occur due to trauma, infectious agents, vitamin C deficiency, and the presence of toxic exposure so that blood came out of blood vessels and caused the hemorrhage. Necrosis began with changes in cell morphology, pycnosis. The next stage was the broken core (karyorrhexis) and the nucleus disappears (karyolysis). Pycnosis could occur due to damage in the cell, including membrane damage followed by mitochondrial damage and Golgi apparatus so that the cell is unable to eliminate water and triglycerides so that it is buried in the cytoplasm of cells [10].

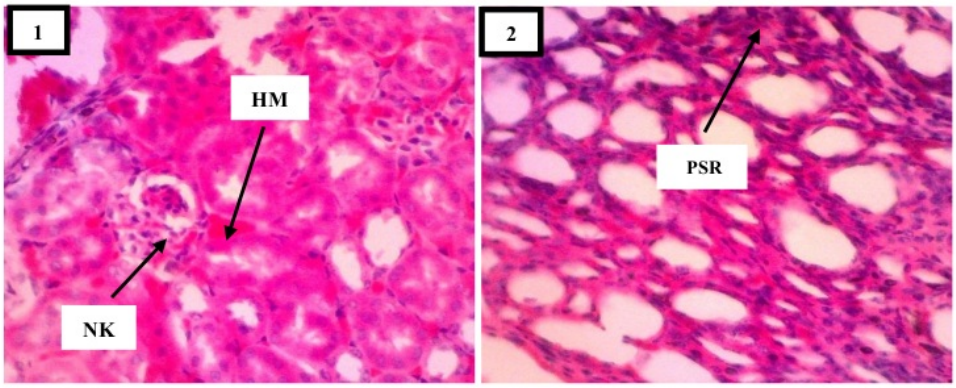

Figure 2. Hemorrhage (1), necrosis (2), and accumulation of inflammatory cells of kidney in KP1 group. Hemorrhage (HM), necrosis (NK), inflammatory cells (PSR). (HE staining, 40×10).

Microscopic observations on KP2 show mild damage, which is almost the same as KP1. The damage was found by necrosis, accumulation of inflammatory cells, hemorrhage, and congestion (figure 3). Congestion was a condition that was accompanied by an increase in the volume of blood in dilated blood vessels in a tool or body part. Congestion occurred through two mechanisms, (1) an increase in the amount of blood flowing to a location or (2) a decrease in the amount of blood flowing from a location. If blood flow to the site increases and causes congestion, it was called active congestion. While passive congestion did not involve an increase in the amount of blood flowing to a location, it was instead a disruption of flow from that location. Anything compressed venules and veins that drain blood from the tissue could cause passive congestion [10].

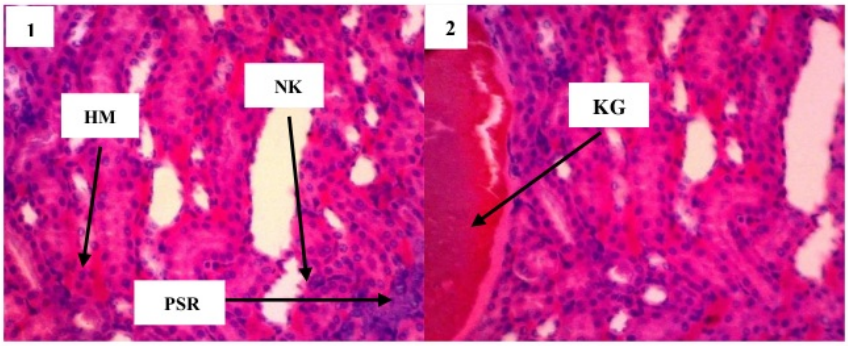

Figure 3. Microscopic observations on KP2 kidney. Hemorrhage **(1),** congestion (2). Necrosis (NK), congestion (KG), hemorrhage (HM), accumulation of inflammatory cells (PSR). (HE staining, 40×10).

Microscopic examination of KP3 showed relatively mild damage, similar to KP1 and KP2, i.e., necrosis, hemorrhage, congestion of blood vessels, and accumulation of inflammatory cells (figure 4). However, it

was found that there was more inflammatory cell infiltration compared to the previous groups. Infiltration is cell entry from outside the tissue. Microscopically, the entire cell infiltration network was characterized by the presence of purple-colored inflammation cells. Infiltration of inflammatory cells in kidney tissue was thought to be related to the body response to some abnormalities due to the activation of thermoregulators. After suffering from inflammation, the body will release various types of biochemical compounds, such as several types of glucocorticoid hormones and cytokines. Cytokines released play an important role in the body's efforts to maintain homeostasis due to inflammation. During inflammation, the cytokine secretion increases; this will increase the inflammatory response. Also, in chronic inflammatory conditions, a protective protein produced due to inflammation (heat shock protein; HSP) becomes out of control, causing several types of cellular proteins to be damaged, the incidence of apoptosis and tissue necrosis also increases [11].

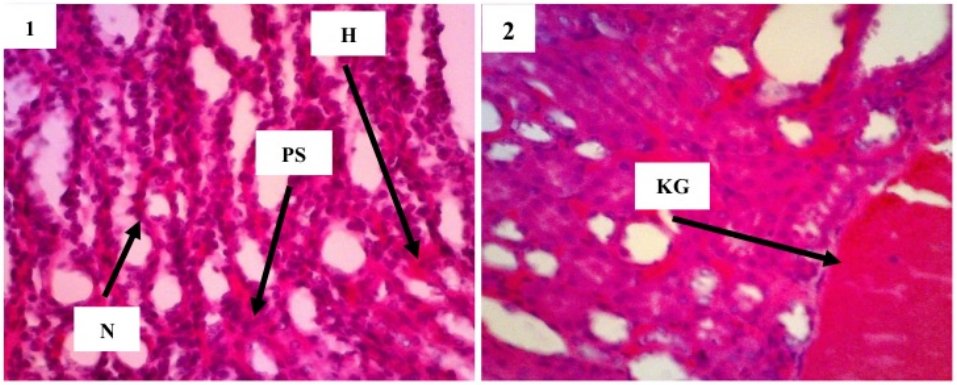

Figure 4. Microscopic observations on KP3 kidney. Hemorrhage (1), congestion (2). Necrosis (NK), congestion (KG), hemorrhage (HM), accumulation of inflammatory cells (PSR). (HE staining, 40×10).

Microscopic observations on KP4 show moderate damage. The kidney showed (necrosis), hemorrhage, accumulation of inflammatory cells, congestion, and narrowing of the lumen. Compared with other groups, hemorrhage and necrosis were more common in this group. These damages were likely to occur due to the administration of toxic doses of erythrosine for a longer time (figure 5). Cell death could be caused by various factors, one of which is hypoxia due to the disruption of the circulatory system by toxic substances that enter. In addition to hypoxia, cell death could also be caused by ischemia. Tubular damage caused by ischemia varies greatly depending on the extent and duration of decreased renal blood flow. Microscopic features of proximal tubular epithelial cells that swell with the granular cytoplasm due to a shift in extracellular water into the cell. This fluid shift occurred because of the presence of toxins that cause changes in electrical charge on the surface of the tubular epithelial cells, changes in the active transport of ions and organic acids, and the ability to concentrate from the kidneys, which ultimately results in damaged tubules, decreased flow. Those conditions might cause the proximal tubule lumen to narrow until it closed [12].

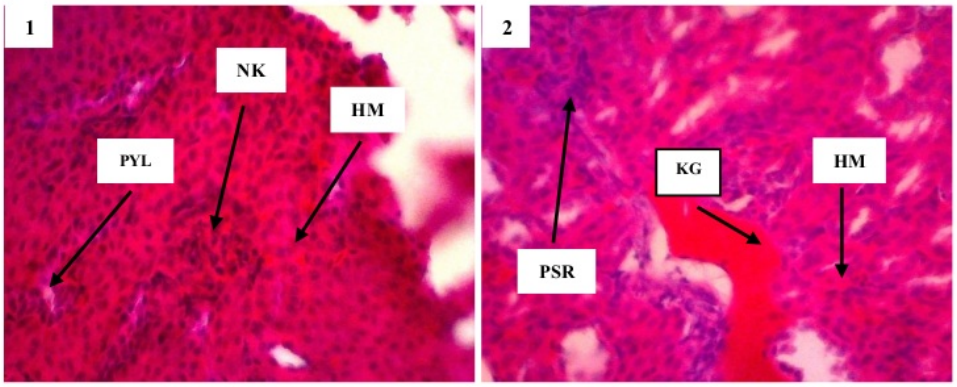

Figure 5. Microscopic observations on KP4 kidney. Hemorrhage (1), congestion (2). Necrosis (NK), congestion (KG), hemorrhage (HM), accumulation of inflammatory cells (PSR), narrowing of the lumen (PYL). (HE staining, 40×10).

Table 2. Glomerular damage after administration of Angkak and erythrosine

Group	Glomerular damage	Observation
KK	Normal	Apparent core, round shape
KP 1	Mild	glomerulus athropy+, capsular enlargement +, hemorrhage +
KP 2	Mild	glomerulus athropy+, capsular enlargement +, hemorrhage +
KP 3	Mild	glomerulus athropy+, capsular enlargement +, hemorrhage +
KP 4	Moderate	glomerulus athropy++, capsular enlargement ++, Hemorrhage ++

- : Normal
+ : 25% cell damage in 3 visual fields
++ : 50% cell damage in 3 visual fields
+++ : 75% cell damage in 3 visual fields

Table 3. Level of tubular damage after administration of Angkak and erythrosine

Group	Tubular damage	Observation
KK	Normal	Apparent core, round shape
KP1	Mild	hydropic degeneration +, fat degeneration +, Hemorrhage +, necrosis +
KP2	Mild	hydropic degeneration +, fat degeneration +, Hemorrhage +, necrosis +
KP3	Mild	hydropic degeneration +, fat degeneration +, Hemorrhage +, necrosis +
KP4	Moderate	hydropic degeneration ++, fat degeneration ++, Hemorrhage ++, necrosis ++

\- : Normal
\+ : 25% cell damage in 3 visual fields
++ : 50% cell damage in 3 visual fields
+++ : 75% cell damage in 3 visual fields

Qualitative data on the level of kidney damage in mice was emphasized in cells undergoing necrosis and changes in the kidney tubules. The results obtained (table 4) generally showed a mild level of damage in KP1, KP2, and KP3, whereas KP4 experienced a moderate level of damage. Cell damage was found as much as 50% in KP4 and 25% in KP1, KP2, and KP3 from observations in the three visual fields conducted. The number of necrotic cells in KP4 reached 50%, while in KP1, KP2, and KP3 was 25%. The necrotic cells occurred due to the binding of toxic substances to cell organelles and giving treatment with toxic doses based on LD50 as well as a long time of administration.

4. Conclusion

The provision of erythrosine with toxic doses caused moderate damage to the kidneys, e.g., lumen narrowing, infiltration of inflammatory cells and the occurrence of cell death (necrosis). In contrast, the administration of Angkak at different doses showed mild damage compared to erythrosine.

References

[1] Yudha A A 2014 Pengaruh pemberian methanil yellow peroral dosis bertingkat selama 30 hari terhadap gambaran histopatologi hepar mencit BALB/C *J. Media Med. Muda*

[2] Susilo A 2014 Pengaruh pemberian methanil yellow peroral dosis selama 30 hari terhadap gambaran histopatologi ginjal mencit BALB/C *J. Media Med. Muda*

[3] Amin M H F, Pidada I B R and Utami C S B 2013 Imunotoksisitas pewarna makanan terhadap histopatologi peyer patch goblet mencit *Bioslogos* **3**

[4] Elok N R and Suryaningsih K 2015 *Bahan pewarna berbahaya yang biasa digunakan pada produk asal hewan dan olahannya* (Jakarta)

[5] Anissa A 2015 *Faktor-Faktor yang Berhubungan dengan Penggunaan Eritrosin dan Rhodamin B pada Pangan Jajanan Anak Sekolah yang Dijual oleh Pedagang di SDN Sekelurahan Pondok Benda Tahun 2015 [Skripsi]* (Jakarta: UIN Syarif Hidayatullah)

[6] Rahmi H 2009 *Studi Hematologis dan Histopatologis Organ pada Tikus yang Diinduksi Kuinin Sebagai Uji Potensi Metabolik Angkak [Skripsi]* (Bogor: Institut Pertanian Bogor)

[7] Anggraini D R 2008 *Gambaran Makroskopis dan Mikroskopis Hati dan Ginjal Mencit Akibat*

Pemberian Plumbum Asetat [Tesis] (Medan: Universitas Sumatera Utara)
[8] Cheville N F 2006 *Introduction to Veterinary Pathology* (USA: Blackwell publishing)
[9] McGavin M D and Zachary J F 2007 *Pathologic Basis of Veterinary Disease* (USA: Mosby Elsevier)
[10] Price S A and Lorraine M W 2006 *Patofisiologi: Konsep Klinis Proses- Proses Penyakit* (Jakarta: EGC)
[11] Huang K L, Wu C P, Chen Y L, Kang B H and Lin Y C 2003 Heat stress attenuates air bubble-included acuted lung injury: Novel mechanism af diving acclimatization *J Appl Physiol* **94** 1485–90
[12] Sari Y E S 2018 Gambaran histologi ginjal tikus wistar yang terpapar msg setelah perlakuan diberikan jus tomat dan diberhentikan perlakuan saja *J. Muhammadiyah Med. Lab. Technol.* **1**

The effect of Angkak and a toxic dose of erythrosine on histopathology of mice (Mus musculus) kidney

ORIGINALITY REPORT

%4	%2	%2	%1
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	F Ali, W S Monica, Y M Adikurniawan, D K Sari, M N Amir. " Fibroblast cell description of provision of sugar and honey in incision wound of domestic cat ( ) ", IOP Conference Series: Earth and Environmental Science, 2020 Publication	%1
2	Submitted to Universitas Brawijaya Student Paper	%1
3	www.ijphrd.com Internet Source	%1
4	www.progressivehealth.com Internet Source	%1
5	Khairun Nisa Berawi, M. Azzaky Bimandama. "The Effect of Giving Extract Etanol of Kepok Banana Peel (Musa Acuminata) toward total Cholesterol Level on Male Mice (Mus Musculus L.) Strain Deutschland-denken-yoken (ddy) Obese", Biomedical and Pharmacology Journal, 2018	<%1

	Publication	
6	www.ncbi.nlm.nih.gov Internet Source	<%1
7	www.ijhhsfimaweb.info Internet Source	<%1

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 5 WORDS
EXCLUDE BIBLIOGRAPHY	ON		