The Comparative Antioxidant Capabilities from Vitamin C and Betanin on The Liver and Renal for Reducing Oxidative Stress Damage: An Animal Experimental Study

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Abstract: Formaldehyde is a hazardous chemical substance that can be found commonly in the environment which has various effects and could induce the production of pro-oxidant substances inside the body. When the concentration of pro-oxidant substances is higher than the concentration of antioxidant substances, it could induce the occurrence of oxidative stress. During oxidative stress, antioxidant play an important role to neutralized harmful effect of free radicals and minimized cellular changes, for examples vitamin C and betanin. The study was conducted to test the differences in the protective effects of vitamin C and betaine on the liver and the kidney after long-term exposure to oxidative stress. The study used rats as experimental media by giving exposure to formaldehyde that is known to induce oxidative stress. The rats were exposed to formaldehyde 6 hours a day for 12 weeks while being given water, vitamin C, and betanin interventions. After 12 weeks of treatments, histopathological examinations were assessed in the liver and kidney organs to compare the protective effects of vitamin C and betanin in those 2 different organs. The results of histopathological examinations revealed that there is a difference in the response to antioxidant therapy between vitamin C and betaine to the liver and kidneys.

Keywords: Antioxidant, Betanin, Formaldehyde exposure, Histopathological, Oxidative stress, Vitamin C

INTRODUCTION

Formaldehyde (FA) is highly reactive chemical through cellular macromolecule, like DNA and protein. In a small concentration, FA is also produced within our body by catalytic enzymatic

processes (Tulpule & Dringen, 2013). When FA get inhaled to our body, it could generate the production of ROS, resulting to cellular oxidative stress. FA exposure occurs more frequently indoors than outdoors. This due to the widespread of FA-containing products, also a lack of awareness and understanding of the negative effects of FA exposure results in the spread of FA intoxication (Kang et al., 2021; M et al., 2016; Tesfaye et al., 2021). Some of the previous studies have discussed about the prevalence of FA exposure, such as the study that was conducted by Driscoll et al. (2015) on the prevalence of workers exposed to FA in Australia. Driscoll, et al reveals out of 4,993 participants, there are 124 participants (2,5%) that exposed to FA, and 67 of them were employed in the technical and commercial sectors (Driscoll et al., 2015). ROS production and changes in the concentration of antioxidant enzymes can both be induced by FA. Protein and lipid peroxidation which can result in oxidative stress are also mediated by FA (Xu et al., 2017). Prolonged oxidative stress due to FA exposure, associated with the activation of the inflammatory mediator TNF-α which is activated by NF-κB and can lead to cellular inflammation (Susilawati et al., 2022). ROS can interfere with the homeostasis of mitochondrial activity, leading to hepatocyte necrosis and tubular inflammation. Overproduction of ROS in the body can alter mitochondrial function, control the expression of pro-inflammatory cytokines, activate immune responses, and trigger inflammatory cascades. Destructing effects of ROS that originate from the mitochondria, endoplasmic reticulum, and peroxysomes are responsible for varies of hepatic degenerative disease. According to some studies, chronic diseases in the liver are almost always accompanied by the production of ROS and its harmful components which eventually cause hepatocellular damage and renovascular instability (Adetuyi et al., 2020; Almunawati et al., 2017; Rafe et al., 2019; Wen et al., 2020). The majority of hepatic diseases are brought on by domestic exposure to toxic substances like FA, which is widely distributed throughout the world. In addition to the exposure method, the liver will primarily process FA, but this process has a detrimental effect on liver condition. FA metabolism will lead to increased acidity, increased CO2 levels, and increased ROS concentration which resulting in hepatotoxicity and nephrotoxicity. Hepatic damage is common due to increased oxidative stress and is responsible for the progression of steatosis to hepatocellular carcinoma (Adetuyi et al., 2020). The neutralizing effects of antioxidants are known as prominent contributing factors to decreasing the impact of oxidative stresses. So far, there isn't much data available yet to show how actually the antioxidant effect, especially from vitamin C and betanin implicates the liver and kidney after common oxidative stress damage by formaldehyde.

This study then sought to determine the actual antioxidant impact of vitamin C and betanin on the hepatorenal histological aspect.

METHOD

This study was a prospective experimental research study that lasts for 12 weeks, from July to September 2023 after receiving approval from the Ethics Committee Faculty of Medicine, Mataram University with registration number 315/UN18.F8/ETIK/2023. The study used a sample of 30 healthy male Wistar rats weighing around 150 - 200grams without any wounds or defects and collected by purposive sampling, divided into 4 groups with 1 healthy control group, 1 negative control group, 1 positive control group, and 3 treatment groups. A single plastic cage measuring 148 cm2 in width and 17.8 cm in height and covered in iron wire nets gives each mouse the same environment. The temperature is kept between 18 - 27°C in room circulation and the intensity of light alternates with dark and light for 12 hours each.

Before treatment, all of the rats are first acclimatized with their environment by regularly giving them standard feeding and water. For the treatment preparation, FA with a concentration of 37% is dissolved until the FA concentration reaches 10%. The replacement group was split into four groups: the healthy control group who doesn't get any treatment, the negative control group receiving 40 ppm FA by inhalation then treated with distilled water, the positive control group receiving 40 ppm FA by inhalation then treated with 3mg Vitamin C / 200 gram rat weight, and the treatment groups receiving 40 ppm FA by inhalation then treated with betanin isolate with tiered concentration about 20, 40, and 80 µg. FA solution is dropped into a previously weighted cotton that has been placed on each cage using micropipe. To prevent upward evaporation and maintain circulation only through the cage's side ventilation, once it has been induced, the mouse cage will be covered with plastic on top of it. The process is left for 6 hours, after which the plastic cage cover will be opened and the induction cotton will be re-weighted. This process is then consistently repeated every day for up to 12 weeks.

Sampling process begins with anesthetic process using Chloroform 10% solution by inhalation to euthanized the rats before performing surgical procedure to obtain liver and kidney organ samples. The surgery was performed, the liver and the kidney were taken, weighted, and stored into cold NaCl 0.9% solution to be cleaned. After that, the liver and the kidney were soaked in 70% alcohol for 24 hours and then stored in a fixative solution (4% PBS). After fixation, embedding, coating the object glass, and tissue slicing were performed to make slides.

After histological assessment were performed, scores of degree dysplasia based on cytological and architectural alterations are used to further evaluate the extent of damage. The histopathological slides were colored with Haematoxylin-eosin (H&E) staining and observed using an Olympus CX41 microscope and Opti lab Pro camera using Opti lab Pro software. The histopathological slides were interpreted based on a score criteria: score 0 = normal; score 1 = mild dysplasia; 2 = moderate dysplasia; 3 = severe dysplasia, based on cytological and architectural changes (Nayak et al., 2019). Data then performed univariate analysis to identify descriptive statistics of dysplasia severities.

Table 1. Grading of dysplasia based on cytological and architectural changes

Grade	Level Involved	Cellular Changes	Architectural Changes
Mild (I)	Lower third	- Cell and nucleus pleiomorphism - Hyperchromatic nucleus	- Basal cell hyperplasia
Moderate (II)	Up to middle third	- Cell and nucleus polymorphism - Anisonucleosis and anisocytosis - Hyperchromatic nucleus - Elevated mitotic abnormality	- Loss of polarity - Abnormal maturation from basal cell to squamous cell - Elevated cellular density - Basal cell hyperplasia
Severe (III)	Up to upper third	- Cell and nucleus polymorphism - Anisonucleosis and anisocytosis - Hyperchromatic nucleus - Elevated mitotic abnormality - Increase nucleus and cell size - Increase size and number of nucleoli	- Nodules - Akantosis - Maturation abnormalities - Elevated cellular density - Basal cell hyperplasia

RESULTS

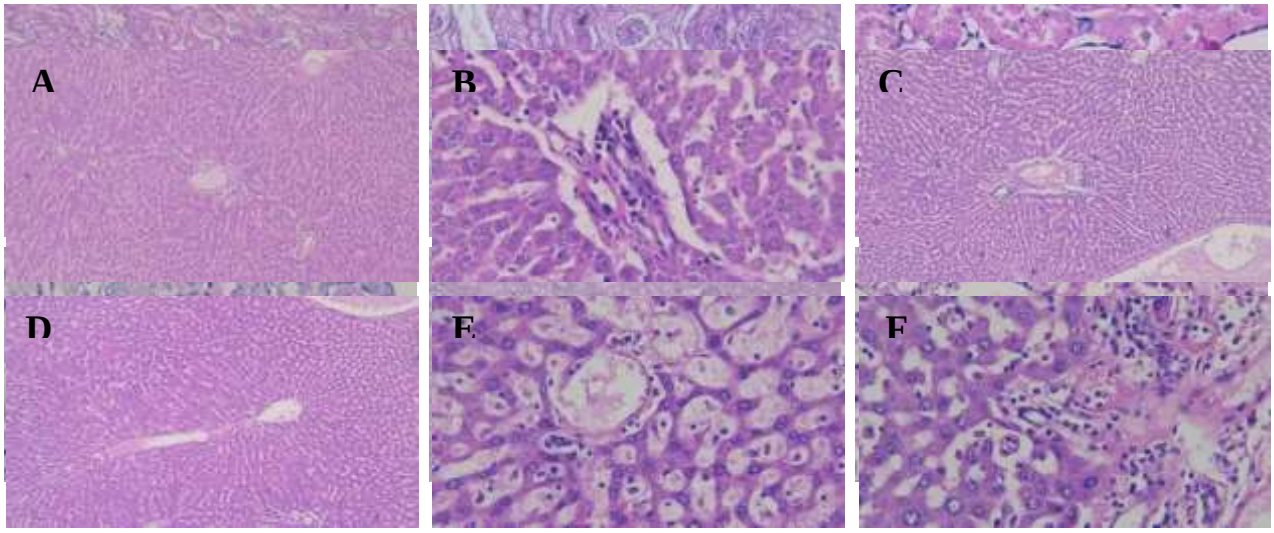
The study used 30 rats divided into 4 groups with a division of 5 rats in the healthy control group, 5 rats in the negative control group, 5 rats in the positive control group, and 15 rats in the treatment groups that were subdivided into 3 sub-groups with 5 rats in the 20 µg betanin subgroup,

5 rats in the 40 µg betanin subgroup, and 5 rats in the 80 µg betanin subgroup. The results of the study showed that the rate of the severity index of dysplasia in the treated mice responded positively as the dose increased.

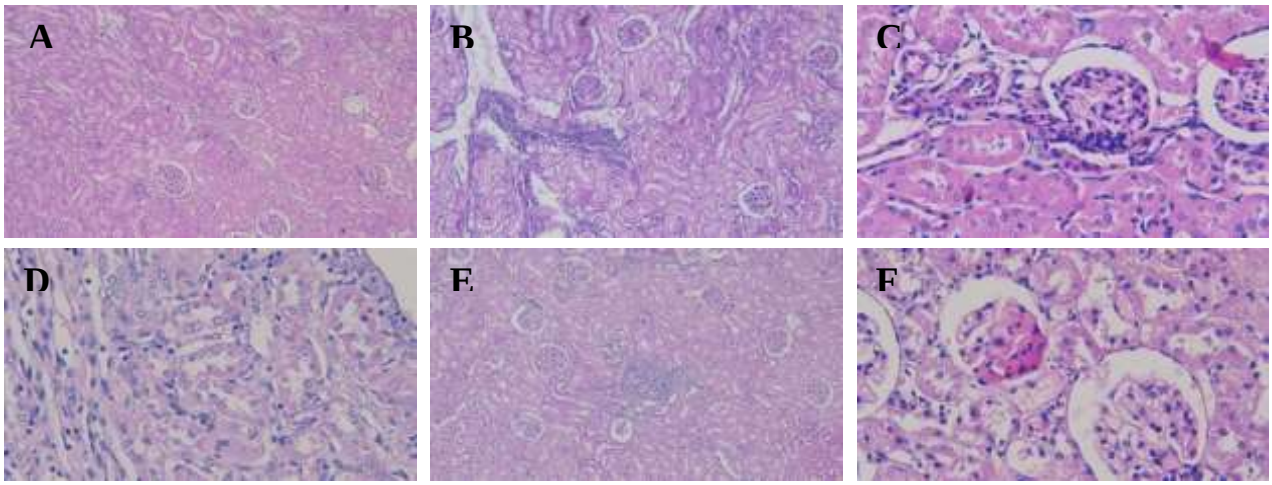
The results of the histopathological examination from the liver showed the protective effect of betanin on the liver. The results showed that increased doses of betanin up to 80 µg had the highest protective effects compared to betanin with a dose of 20 and 40 µg or with vitamin C at a dose of 3 g/200mg rat weight. While in the kidneys, the protective effect is more visible from vitamin C than from betanin at a dose of 80 µg

Table 2. Result summary grading of dysplasia on liver and kidney from each group based on histopathological examination.

Summary of dysplasia severity from each group		
Group	**Average score**	
	Liver	**Kidney**
Healthy control	1	0
Negative control	1.2	2.2
Positive control	2	1.4
Treatment 1	2	2
Treatment 2	1.8	1.8
Treatment 3	1.4	1.6



Picture 1. Histopathological appearance of liver: A. Healthy control; B. Negative control; C. Positive control; D. 20µg betanin; E. 40µg betanin; F. 80µg betanin



Picture 2. Histopathological appearance of kidney: A. Healthy control; B. Negative control; C. Positive control; D. 20µg betanin; E. 40µg betanin; F. 80µg betanin

DISCUSSION

Formaldehyde is a substance with high toxicity, genotoxic, and carcinogenic to tissue organ (Ramos et al., 2017). This is because the exposure to formaldehyde causes widespread DNA damage and triggers the onset of liver organ attacks and leukemia (Reingruber & Pontel, 2018). In a previous study conducted by Wedayani et al. showed that exposure to formaldehyde at a dose of 40 ppm caused the occurrence of severe degree dysplasia in the nasopharynx of wistar rats. Theoretically, persistent exposure to FA over a long period of time can induce the occurrence of oxidative stress characterized by increased pro-oxidant markers and decreased antioxidant markers. (Tesfaye et al., 2021; Xu et al., 2017). If it lasts for a long time, it can lead to damage and dysfunction of the tissue. In conditions of oxidative stress where the amount of antioxidants is much

lower than pro-oxidants, it will cause imbalance of body functions in regulating free radicals, radical attacks, damage to proteins, lipids, and nucleic acids resulting from this activity can imply the loss of energy for metabolism, cellular communication, transport function, proteosomal degradation, and other cellular functions, so based on existing theories, excessive accumulation of free radical can allow to be one of the keys to the occurrence of insulin resistance, diabetes, and cardiovascular disease (Mahjoub & Roudsari, 2012; Zhang et al., 2018). The tissues that most influence the metabolic process are the liver, muscle, and adipose tissue. When in a state of oxidative stress, changes in metabolic function will occur, with an increase in mitochondrial NADH (mNADH) and ROS. Physiologically, when there is an elevation of the pro-oxidant mediator in the blood, then the formation of endogenous ROS in the body will be inhibited. The process of inhibition of ROS formation occurs by inhibiting the formation of mNADH by inhibiting the function of insulin and inhibits the entry of energy substrates (Mahjoub & Roudsari, 2012; Tesfaye et al., 2021). This will imply the condition of tissues that experience deficient nutritional status. Each cell in the body of a multicellular organism has the ability to respond to metabolic energy deficiencies in either physiological or pathological conditions because it has cells that are specialized in detecting nutrient deficiencies. On this basis, rats who experience oxidative stress will have a higher appetite, but their body conditions cannot distribute glucose substrate optimally and can end up in a metabolic syndrome that can imply to the liver (Caro-Maldonado & Muoz-Pinedo, 2011; M et al., 2016; Mahjoub & Roudsari, 2012). The accumulation of excess glucose will also lead to the occurrence of glycosylation processes. The glycosylation process is initiated by the presence of a chemical reaction between the carbon group of glucose with the group of proteins-free nucleophilic amino acids that will lead to the formation of an unstable Schiff function group. Physiologically in the body, the unstable Schiff function group will be converted into a more stable Amadori product, but in too many amadori products will undergo oxidation, dehydration, polymerization, and cross-reaction into the Advance Glycation End-product (AGE) which is highly toxic at the cellular level, and implicates the activation of inflammatory mediators and the formation of ROS (Kim et al., 2017).

In this study, rats were induced with FA which has been internationally recognized by the IARC as a carcinogenic agent. Cells that mutate into carcinomas depend heavily on the presence of glucose as their primary source of nutrients. In a study conducted by Susilawati et al. (2022) stated that with an induction of 20 ppm of 10% FA over 16 weeks caused rats to develop nasopharyngeal dysplasia. In this study, the treatments used were inductions of 40 ppm FA at a concentration of 10% over 12 weeks for the negative control group, positive control group, and the

treatment groups, so the index of cellular severity experienced in rats should be higher. The result of this study revealed the beneficial effects of vitamin C and betanin in liver and kidney. According to this study, compared to vitamin C at a dose of 3g/200 mg rat weight, betanin at doses of 40 and 80 µg is more advantageous as an antioxidant to lessen radical damage on the liver. Conversely, compared to up to 80 µg of betanin, vitamin C at a dose of 3g/200mg rat weight has a more beneficial effect on the kidney in terms of reducing radical damage.

Betanin is a plant pigment that belongs to the group of betaines. It is usually applied to food, cosmetics, and pharmaceuticals. Betanin in foods or extracts rich in betanin are known to have a variety of potential and health benefits, including inhibiting the activity of free radicals, preventing DNA damage and inducing the formation of antioxidants, gene-regulating activity, and anti-inflammatory activity. One study noted that a diet of red beet extract in mice could induce endogenous antioxidant defense mechanisms and inhibit DNA damage to lymphocytes and hepatocytes (Esatbeyoglu et al., 2015). A study has been conducted to look at the effects of the use of BHE and betanin from red beet extract on colorectal cancer cells (Caco-2, ATCC, HTB-37 and HT-29, ATCc, H TB-38). In this study, it has been shown that BHE with concentrations of 100 µg/mL inhibits the growth of HT-29 and Caco-2 cell lines significantly. In addition, the lowest concentration of Betanine and BHE (in succession 60 µg / mL and 100µg/) significantly inhibit the rate of cancer cell growth (Saber et al., 2023). Other research has shown that the antioxidant and anti-inflammatory activity of betanins can weaken the main mechanisms of atherogenesis and cardiovascular disease (Silva et al., 2021). A study has been conducted to look at the benefits of the use of 25 mg/kg and 100mg/kg betaine doses against the kidneys of rats induced by 20 mg/ kg paraquat. Histological evaluation suggests that paraquat injection causes acute nephrotoxicity in the paraquate group. This was characterized by tubular and glomerular degeneration, bleeding, and necrosis. Unlike the group, other groups given betaine during the study had good glomerulus and tubular structural defences, as well as reduced bleeding and significantly lowered histological scores. The study also assessed the effect of betanine on oxidative stress caused by paraquat. The results showed a decrease in oxidative stress due to the known paraquat through the reduction of TBARS, and a significant increase in SOD and CAT (Tan et al., 2015).

Vitamin C is one of the antioxidants that plays a key role in the body's metabolism. In the literature by Jia (2015) has summarized the latest evidence that suggests a link between vitamin c intake and a decreased risk of renal cell carcinoma. Research by Mehany (2023) has assessed the improvement in kidney condition due to induction of DENA after administration of vitamin C and

curcumin. One of the variables studied is the histopathological picture of kidney tissue. In the group given induction of DENA without vitamin C and curcumin showed changes in histopathological picture such as dilated and congested blood vessels, perivascular edema and hyalin degeneration of the blood vessel wall, degenerative changes in kidney tubules, apoptosis of cortical tubules epithelium, and changes in glomeruli image (Mehany et al., 2023).

CONCLUSION

According to this research, the antioxidant effects from vitamin C and betanin contributes to the reduction of radical damage due to FA induction. There is a difference in the therapeutic effect produced on the liver and kidney organs with the antioxidant effect of betanin being stronger in the liver while the antioxidant effects of vitamin C are stronger in the kidneys. These findings demonstrate that both of vitamin C and betanin has a prominence protective effect on different tissues and organs either it was the liver or the kidney.

Conflict of Interest

The author reports no conflicts of interest in these studies.

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