

Phytochemical Screening and Antioxidant Activity of Strawberry Juice (*Fragaria ananassa* Duchesse) Against Urea Level, Creatinin, and Enzyme Catalase Activity In Isoniazid-Induced Wistar Male Rats

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ABSTRACT

Kidney are vital organ for human. Isoniazid is an antituberculosis drug that causes multilobular necrosis and acute tubulointerstitial nephritis (ATIN). Isoniazid nephrotoxicity in kidney is able to trigger oxidative stress through the formation of reactive oxygen species (ROS). ROS increase can causes damage to the kidney so that urea and creatinine level increase in the blood and can be used as a marker of decreased kidney function. Excessive free radicals can cause a decrease in endogenous antioxidant activity, namely the catalase enzyme. This condition can be overcome by given exogenous antioxidants such as strawberry juice (*Fragaria ananassa* Duchesse). This study aimed to determine the effect of strawberry juice treatment against urea and creatinine levels in male Wistar rats Isoniazid-induced for 14 days divided into groups, namely normal control, negative control, positive control, strawberry juice dose of 3 g/kgBW, 6 g/kgBW, and 9 g/kgBW. Data were collected on 1st, 15th, and 29th day. The results of the study concluded that strawberry juice had an effect in reducing levels of urea, creatinine and catalase enzyme activity in isoniazid-induced rats with an effective dose of 3g/kgBW.

Keywords: Catalase Enzym, Creatinin, Isoniazid, Strawberry Juice, Ureum

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BACKGROUND

Kidney are vital organ for human. Kidney have a role for the body as the disposal of Non-protein Nitrogen compound (NPN), regulator of body fluid balance, regulator of electrolyte balance, and regulator of acid-base balance (Verdiansah, 2016). Impaired kidney function occurs when the body fails to maintain metabolism and balance of fluids and electrolytes resulting in retention of urea and other nitrogenous wastes in the blood (Brunner & Suddarth, 2001).

Isoniazid is the most important anti-tuberculosis (OAT) drug for the prevention and treatment of tuberculosis (TB), either as monotherapy or in combination with other TB drugs (Rahman, 2013). There are quite a lot of side effects caused by isoniazid, one of which is can cause multilobular necrosis (Katzung, 2008). Isoniazid also has an effect on the kidney, called acute tubulointerstitial nephritis (ATIN) (Appel & Bhat; 2006 Baker & Pusey 2004). Isoniazid nephrotoxicity in the kidney is capable to inducing oxidative stress through the formation of reactive oxygen species (ROS). The increase in reactive oxygen species will cause damage to the kidney (Sharma, 2012).

Investigation of kidney function damage can be seen from the levels of substances that are filtered by the glomerulus by the blood, such as creatinine and urea. If there is a decrease in renal function and the GFR rate decreases, the creatinine and urea levels filtered by glomerulus will decrease, so that their levels in the blood will increase. Increased levels of urea and creatinine in the blood can be used as a marker of decreased kidney function (Guyton and Hall, 2008). High serum urea and creatinine levels can occur in chronic renal failure and hemodialysis therapy does not reflect a decrease in serum urea and creatinine levels back to normal (D G A Suryawan et al, 2016).

In addition, excessive free radicals can also cause a decrease in endogenous antioxidant activity, including catalase enzyme. Catalase is an enzyme that converts hydrogen peroxide into water and oxygen molecule. Hydrogen peroxide (H₂O₂) is one of the free radical. Excessive H₂O₂ is a toxic source of various diseases because it can cause tissue damage (Untari, 2014). Catalase enzyme plays a role in controlling the concentration of H₂O₂ by catalyzing H₂O₂ so that it is less even non-toxic (Apriana et al., 2016: 36).

An effort to overcome health problems, especially kidney, are by using antioxidants (Kumalaningsih, 2007). One of the exogenous antioxidants of natural substance is strawberry. Strawberry is a fruit that is often consumed by the public and easy to find out. Strawberry have a high content of antioxidants and phenolic compounds (Oszmianski et al., 2009). The main class of phenolic compounds is represented by flavonoid (mainly anthocyanin, with flavonol acting as minor contributor), followed by hydrolyzed tannin (ellagitannin and gallotannin) and phenolic acid (hydroxybenzoic and hydroxycinnamic acid), and condensed tannin (proanthocyanidin) (Kahkonen et. al., 2001; Aaby et al., 2005).

Based on the description above, can be assumed that strawberry have antioxidant activity as a nephroprotector. This study was done to determine the effect of strawberry juice (*Fragaria ananassa* Duchesne) treatment on urea levels, creatinine, and catalase enzyme activity in Isoniazid-induced male rats at dose of 200mg / kgBW.

METHODS

Ingredients. Ingredients that used were strawberry fruit, male Wistar rats, aquadestilata, isoniazid from PT Phapros, Curcumin p.a, CMC Na, Picric acid, Sodium hydroxide, Disodium phosphate, Tris buffer (pH 7.60) , ADP, α -ketoglutarate, Urease, GIDH, Sodium azide, NADH, NaOH, HCl, 50 mM potassium phosphate buffer (pH 7.0), hydrogen peroxide (H₂O₂ 10 mM), and 32.4 mM ammonium molybdate.

Equipment. Equipment that used in this study were scale (digital and analytical), juicer, beaker glass, glass funnel, stirring rod, centrifuge tube, capillary tube, measuring cup, animal cage, injection syringe with blunt tipped needle, centrifuge (Gemmy centrifuge PLC-05), micropipette, asbestos, stativ, thermometer, clamp, UV-VIS Spectrophotometer, and Spectrophotometric Mikrolab 300.

Making Strawberry (*Fragaria ananassa* Duchesse) Juice. Fresh fruit that had been sorted, washed, cleaned from the leaves then weighed according to the calculation. Put the strawberry fruit into the juicer and extract the juice from the fruit. Juice then collected in a container. Volume of strawberry (*Fragaria ananassa* Duchesse) juice measured. Juice in a measuring cup to determine volume that obtained. Put in into a bottle that protected from sunlight and stored in the refrigerator.

Strawberry (*Fragaria ananassa* Duchesse) Juice Phytochemical Screening. The preliminary tests that have been done include:

1. Flavonoids

Strawberry juice (*Fragaria ananassa* Duchesse) was put in a test tube, added with Mg powder and 1 ml of concentrated HCl, then added amyl alcohol, shaken vigorously and allowed to separate, the changes were observed (Harborne, 1987: 73). The presence of flavonoid compounds was indicated by the formation of a red or orange color in amyl alcohol compound (Endarini, 2016).

2. Tannin

Strawberry juice (*Fragaria ananassa* Duchesse) was added to a test tube with 10% NaCl added, then filtered and the filtrate was taken. The filtrate was divided into two, called filtrate A and B. Filtrate A was added with 1% gelatin. Positive result if a white precipitate was formed. Filtrate B added with 10% FeCl₃ solution. Positive results occurred in dark green or bluish green (Endarini, 2016).

3. Saponin

Strawberry juice (*Fragaria ananassa* Duchesse) was put in a test tube, added 10 ml of hot water, cooled and then shaken vigorously for 10 seconds. The reaction is positive if a stable foam was formed for not less than 10 minutes with 1 to 10 cm high. With the addition of 1 drop of 2 N hydrochloric acid, the foam did not disappear (MOH, 1979: 170).

4. Alkaloids

Strawberry juice (*Fragaria ananassa* Duchesse) was put in a test tube then mixed with 1 ml of 2N HCl and 9 ml of hot aquadest. The solution was heated for 2 minutes, then cooled and filtered. Filtrate was put into 2 test tubes. The first tube was added with Dragendorff reagent and the second tube was added with Mayer reagent (Depkes RI, 1995). The positive results of alkaloids were indicated by the formation of a reddish brown precipitate when Dragendorff reagent is added and a white precipitate is formed when the Mayer reagent is added (Endarini, 2016).

5. Steroids and Triterpenoids

Strawberry juice (*Fragaria ananassa* Duchesse) was put into a test tube mixed with ether, then filtered and the filtrate was taken. The filtrate was evaporated in an evaporator cup until a residue was obtain. The residue was added with 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid. The presence of terpenoid was indicated by the appearance of a red color while the presence of steroid was indicated by the appearance of a blue color (Endarini, 2016).

The Confirmation Test or TLC of Strawberry Juice (*Fragaria ananassa* Duchesne) includes:**1. Flavonoids**

Eluent that used were n-butanol: glacial acetic acid: water (4: 1: 5) and then separated to take the n-butanol phase. The plate was sprayed with ammonia vapor. The formation of a brownish yellow color indicates the presence of flavonoids (Robinson, 1995: 211).

2. Tannin

Eluent that used were toluene: ethyl acetate (3: 1). The plate were sprayed with FeCl₃. The formation of a green-black color indicates the presence of tannin compounds (Robinson, 1995: 78)

3. Saponin

The eluent used were chloroform: methanol: water (64:50:10). The plate were sprayed with the sulfuric acid anisaldehyde then oven at 100 °C for 5-10 minutes. Green or blue color in UV light indicated the presence of saponin compounds (Nuria et al., 2009).

4. Alkaloids

Eluent used were ethyl acetate: methanol: water (100: 13,5: 10). The plate was sprayed with dragendorff reagent. Alkaloid compounds occurred a brown or orange color (Arifin et al., 2006).

5. Triterpenoid

Eluent used were toluene : ethyl acetate (93:7) and detected using anisaldehyde-sulfuric acid (oven 110 °C for 5-10 minutes). formation of red, purple, dark purple, green, blue, red spots indicated positive triterpenoid compound (Hayati et al., 2012).

Experimental Animal Treatment. This research was used 30 rats and divided into 6 groups, called group I (normal control), group II (treated with CMC Na 0.5%), group III (treated with Curcumin 50 mg/kgBW), group IV (treated with strawberry juice at dose 3g/kgBB), group V (treated with strawberry juice at dose 6g/kgBB), and group VI (treated with strawberry juice at dose 9g/kgBB). Group I was given standard feed (bio rat) and drink, while groups II, III, IV, V, and VI were given 200 mg/kgBW of isoniazid induction for 14 days. Furthermore, treatment was given according to each group for 14 days. Measurement of urea and creatinine levels was carried out on day 1st, day 15th and day 29th. The data obtained were analyzed using SPSS version 19.

Measurement of the enzyme catalase activity. 100 µl of blood serum was measure and 2000 µl of 50 mM potassium phosphate buffer (pH 7.0) containing 10mM of hydrogen peroxide (H₂O₂) was added (Iwai et al., 2002). The solution was incubated at 37 °C for 1 minute and then added 1.0 ml of 32.4 mM ammonium molybdate [(NH₄)₆ Mo₇O₂₄.4H₂O)]. The absorbance was measured using a UV-Vis spectrophotometer at λ 485 nm.

RESULT

This research is an experimental study with a Pre and Posttest Randomized Control Group Design. The research was done from February 2020 to July 2020. The research was done at the Pharmacology Laboratory College of Pharmacy foundation Semarang. This research had received an ethical approval number 094/CN/SW/KEPK-STIFAR/EC/I/2020

Table 1. Phytochemical Screening and TLC result

Compound	Result	
	Fresh Juice	TLC
Flavonoid	+	Rf 1 : 0,76 Rf 2 : 0,59
Alkaloid	+	Rf : 0,73
Tannin	+	Rf : 0,39
Saponin	+	Rf : 0.48
Steroid/ Triterpenoid	-	Rf : -

The result of measuring the levels of urea, creatinine, and catalase enzyme activity can be seen in table 2,3, and 4.

Table 2. Mean \pm SD of Decrease Measurement Test and the Decrease Percentage of Urem Levels

Group	Mean \pm SD (mg/dL)			Decrease Percentage (%)
	1 st Day	15 th Day	29 th Day	
Normal control	30,06 \pm 8,44	28,46 \pm 2,33	33,92 \pm 7,29	-19,60 \pm 24,58
Negative control (CMC Na 0.5%)	28,08 \pm 4,75	45,74 \pm 8,48	49,80 \pm 10,13	-8,71 \pm 6,80
Positive control (Curcumin 50mg/KgBW)	36,38 \pm 4,00	35,28 \pm 6,96	28,52 \pm 8,54	18,10 \pm 23,00
Strawberry Juice 3g/KgBW	33,56 \pm 3,51	42,58 \pm 6,20	17,12 \pm 7,35	59,10 \pm 16,64
Strawberry Juice 6g/KgBW	31,92 \pm 4,34	48,40 \pm 7,03	21,68 \pm 9,51	54,64 \pm 20,98
Strawberry Juice 9g/KgBW	30,16 \pm 7,06	38,62 \pm 8,66	19,58 \pm 14,47	48,94 \pm 34,72

Table 3. Mean \pm SD of Decrease Measurement Test and Decrease Percentage of Creatinine Levels

Group	Mean \pm SD (mg/dL)			Decrease Percentage (%)
	1 st Day	15 th Day	29 th Day	
Normal control	0,67 \pm 0,22	0,52 \pm 0,27	0,51 \pm 0,18	-3,75 \pm 18,95
Negative control (CMC Na 0.5%)	0,51 \pm 0,17	0,53 \pm 0,08	0,50 \pm 0,07	1,97 \pm 25,17
Positive control (Curcumin 50 mg/KgBW)	0,56 \pm 0,24	0,45 \pm 0,06	0,41 \pm 0,03	7,17 \pm 17,95
Strawberry Juice 3g/KgBW	0,72 \pm 0,19	0,52 \pm 0,15	0,51 \pm 0,12	-1,65 \pm 21,83

Strawberry Juice	0,53	±		
6g/KgBW	0,59 ± 0,19	0,18	0,33 ± 0,12	36,42 ± 20,73
Strawberry Juice	0,58	±		
9g/KgBW	0,43 ± 0,09	0,17	0,44 ± 0,09	20,22 ± 24,74

Table 4. Mean ± SD of Increase Measurement and Increase Percentage of Catalase Enzyme Activity

Group	Mean±SD (U/ml)			Increase Percentage (%)
	1 st Day	15 th Day	29 th Day	
Normal control	48,31 ± 5,62	47,82 ± 5,24	50,22 ± 5,27	5,14 ± 4,80
Kontrol Negatif (CMC Na 0.5%)	44,28 ± 7,12	30,49 ± 2,22	32,58 ± 2,16	6,91 ± 1,58
Positive control (Curcumin 50mg/KgBW)	44,65 ± 5,07	32,71 ± 3,65	45,41 ± 3,96	39,20 ± 4,84
Strawberry Juice 3g/KgBW	41,23 ± 5,26	30,46 ± 6,58	39,45 ± 6,80	30,42 ± 6,78
Strawberry Juice 6g/KgBW	45,97 ± 2,50	33,66 ± 3,28	46,18 ± 4,20	37,29 ± 2,42
Strawberry Juice 9g/KgBW	42,40 ± 7,31	32,18 ± 6,78	49,35 ± 6,66	55,54 ± 13,92

DISCUSSION

This study aim to determine the effect of giving and effective dose of strawberry juice (*Fragaria ananassa* Duchesse) on urea and creatinine levels in Isoniazis-induced male Wistar rats. Sample used were strawberry (*Fragaria ananassa* Duchesse).

The orientation of strawberry juice was done and result volume of juice was 20.38 ml for 30g of strawberries. Preliminary test (phytochemical screening) and affirmation test (Thin Layer Chromatography) of strawberry juice was done. The purpose of the phytochemical screening test was to determined the secondary metabolite compounds contained in strawberry juice. From the test results, it is known that strawberry juice contains flavonoid, alkaloid, tannin and saponin. The most common compounds in strawberry are ellagitannin, anthocyanin and flavonol. Flavonol that identified in strawberry are derivative of quercetin and kaempferol (Aaby et al., 2007).

Pharmacological activity tests was done on testing animal to determine the pharmacological activity of strawberry juice in reducing urea and creatinine levels and increasing the activity of the enzyme catalase. Normal urea levels are 12.3-24.6 mg / dL (Mary and Charles, 2008). Normal creatinine levels are 0.2-0.5 mg / dL (Mary and Charles, 2008).

To determine the antioxidant activity of strawberry (*Fragaria ananassa* Duchesse) juice on decreasing levels of urea and creatinine, and increasing activity of the enzyme catalase seen from the percent decrease in levels of urea, creatinine, and percent increase in catalase enzyme activity. The decrease percentage of urea, creatinine, and increase percentage in catalase activity that obtained were tested for normality and homogeneity test. The normality test shows that the data is normally distributed ($p > 0.05$) and the homogeneity test shows that the data is homogeneously distributed ($p > 0.05$).

A parametric test for the percent reduction in urea, creatinine, and increase of catalase activity was performed using the One-Way Anova test with confidence level of 95%. The results of the One-Way Anova test for the percentage of reduction in urea and creatinine levels showed a difference in urea levels between groups with a value of $p = 0.000$ and a decrease in creatinine levels with a value of $p = 0.007$. The increase activity of catalase enzyme showed difference in urea levels between groups with a value of $p = 0.000$. A Post Anova test was performed, called Post-hoc LSD test to see the significance value between groups.

Post-hoc LSD test results In the positive control group compared with the strawberry juice group (*Fragaria ananassa* Duchesse) at doses of 3g / kgBW, 6g / kgBW, and 9g / kgBW, the result were not significant. This explains that strawberry juice (*Fragaria ananassa* Duchesse) doses of 3g / kgBW, 6g / kgBW, and 9g / kgBW were able to reduce levels of urea and creatinine in Isoniazid-induced rats by comparable to the positive control (Curcumin) at a dose of 50 mg / kg in rats. This is the base for selecting an effective dose of strawberry juice (*Fragaria ananassa* Duchesse) that the dose of 3g / kgBW is an effective dose to reduce urea and creatinine levels in rats.

Isoniazid used as an inducer can damage the kidney. The mechanism of isoniazid in damaging the kidney begin with the change of isoniazid by N-acetyltransferase2 (NAT-2) to acetyl isoniazid, which will then be hydrolyzed to acetylhydrazine. Acetylhydrazine with the help of cytochrome P4502E1 enzyme converted to N-hydroxyacetyl hydrazine, which will further be converted into acetyldiazine. Acetyldiazine which is an intermediate product will be change into free radical (Tostmann et al., 2008).

One of the cause of increased levels of urea and creatinine is free radical. Free radicals are a nephrotoxic mechanism of isoniazid, and antioxidants can be protecting against free radical cause nephrotoxic and ROS (reactive oxygen species) induce in the kidney. The increase in free radical and ROS will cause cell death where the content of the cell that come out will bind to the fibronectin protein in the tubular lumen. This will cause a blockage in the form of a cylinder so that urea and creatinine cannot be excreted properly (Michael, 2013). In the research of Muhyi et al., (2014) it was proven that the use of isoniazid can cause an increase in creatinine and urea levels.

In addition, oxidative stress due to increased free radical production causes a decrease in endogenous antioxidants in the liver such as catalase, superoxide dismutase, and glutathione peroxidase (Zalukhu et al., 2016). Free radicals that formed by oxygen molecule called Reactive Oxygen Species (ROS). ROS will be converted by the enzymatic antioxidant SOD to H_2O_2 and then the catalase enzyme will convert H_2O_2 to H_2O and O_2 . Continuously exposure of free radicals will cause enzyme activity decrease, so that H_2O_2 becomes toxic hydroxyl radicals. So that exogenous antioxidants are needed to neutralize free radical (Sy et al., 2015). Antioxidant can stop the production of Reactive Oxygen Species (ROS) which will cause cell damage such as obstruction in the urinary tract (Shah, 2014).

In this study, it was shown that strawberry juice (*Fragaria ananassa* Duchesse) was able to reduce levels of urea and creatinine and increase the activity of the enzyme catalase. This can occur because strawberry juice (*Fragaria ananassa* Duchesse) contains flavonoid, alkaloid, tannin, saponin, and vitamin C compound. In addition, strawberry (*Fragaria ananassa* Duchesse) were assumed to contain marker quercetin compounds (Aaby et al., 2007). These compounds have a role as antioxidants.

Flavonoids have antioxidant activity that can inhibit lipid peroxidation by reducing free radicals and increasing intracellular concentrations of glutathione (Karimi et al., 2009).

The mechanism of flavonoids as a breakdown of calcium oxalate crystals, namely flavonoids will bind with calcium to form complex compounds into Ca-flavonoids (Susilo, et al. 2018)

Alkaloid class compounds can be potent as antioxidants. Alkaloid compounds have the ability to stop free radical chain reactions, alkaloids also play an important role in protecting cells from the effects of radiation and drug toxicity (Yuhermita and Juniarti, 2011). In addition, alkaloid also have been shown to have a strong inhibitory effect on lipid peroxidation in tissue isolation by increasing the enzyme superoxide dismutase (SOD). This inhibition will be able to cause the return of Na⁺ and H₂O function to the cells so that kidney function return to normal (Shelkea et al., 2011).

Tannins have the ability to bind free radicals, tannins are very effective as electron donors or hydrogen atoms, this happens because tannins have hydroxyl groups and conjugated double bonds that allow electron delocalization (Hagerman, 1998). Vitamin C and tannin act as free radical scavengers that bind directly to free radicals or toxic drug metabolites (Hassanin et al., 2013) (Gulcin et al., 2010).

Saponins have anti-oxidative and radical-binding properties by forming hydroperoxides as intermediates and can contribute hydrogen to radical compounds thus ending the radical chain reaction (Xiong et al., 2012).

Quercetin is able to improve the redox state of cells and increase the expression of c-glutamethylsyn synthesis, which is a rate-limiting step in the synthesis of antioxidant glutathione peroxidase (GSH-Px). Increased activity of T-SOD, GSH-Px will have a protective effect on the glomerulus and proximal tubule which causes a decrease in tissue damage mediated by free radical reactions (Myhrstad et al, 2002).

CONCLUSION

Strawberry juice (*Fragaria ananassa* Duchesse) has antioxidant activity which act as a nephroprotector. The treatment of strawberry juice (*Fragaria ananassa* Duchesse) can reduce levels of urea and creatinine and increase the activity of catalase enzyme in Isoniazid-induced rats. The effective dose of strawberry juice (*Fragaria ananassa* Duchesse) which can reduce urea and creatinine levels and increase catalase enzyme activity in Isoniazid-induced rats is 3g / kgBW.

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