

# Kidney Evaluation in Hyperuricemia Rats Treated with Green Tea Leaves (*Camellia sinensis* L.) Extract

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## Abstract

Uric acid is an oxidation product of the xanthine oxidase enzyme found in extracellular fluid, and when it exceeds, uric acid will build up and cause hyperuricemia. TNF- $\alpha$  is released by epithelial cells and mesangial cells when inflammation occurs and causes apoptosis in epithelial cells, causing damage to kidney structures and initiating acute kidney poisoning. Green tea extract (*Camellia sinensis* L.) contains many antioxidants, especially flavonoids with potent antioxidant properties such as lipid peroxidase and free radical absorbers, inhibiting xanthine oxidase. This study expresses the potential of green tea extract on kidney repair caused by HUA. Twenty-four male albino rats (175-225 g) of Wistar strain being fed a high purine diet in 60 consecutive days and divided into six groups randomly, I: negative control, II: positive control, III: allopurinol, IV: green tea extract 150mg of body weight, V: green tea extract 300mg of body weight, and VI: green tea extract 600mg of body weight. Treatment was done for 14 days and measured by total creatinine levels, malondialdehyde levels, and kidney histopathology. The statistical analysis using One Way ANOVA and Post Hoc Tukey analysis by SPSS 23.0 proved that green tea extract with a dose of 600 mg/kg of body weight green could lower levels malondialdehyde of the kidney as much as 58.85% ( $p < 0.01$ ), decreased creatinine level by 24.5% ( $p > 0.05$ ), and improved kidney histopathology. This study proved that green tea extract is a promising alternative for hyperuricemia while improving kidney tissues and lowering malondialdehyde and creatinine levels.

**Keywords:** *Camellia sinensis* L.; hyperuricemia; kidney creatinine; kidney histopathology; kidney malondialdehyde.

**Abbreviations:** UA, Uric Acid; HUA, Hyperuricemia; ROS, Radical Oxygen Species; NF $\kappa$ B, Nuclear Factor kappa-light-chain-enhancer of activated B cells; GTE, Green Tea Extract; ALP, Allopurinol; MDA, Malondialdehyde; BW, body weight; XOD, Xanthine Oxidase; URAT1, Urate Transporter 1; OAT1, Organic Anion Transporter 1; OAT3, Organic Anion Transporter 3; PBS, Phosphate Buffered Sucrose; PFA, Paraformaldehyde

## INTRODUCTION

Uric acid (UA) comes from endogenous sources and exogenous sources. Endogenous sources come from protein and nucleoprotein breakdown products, while exogenous sources come from purine foods. The body's average UA levels are 3.4-7.0 mg/dL in men and 2.4-6.0 mg/dL in women, and 75% of the urate will be excreted in the urine (Lippi et al., 2008). UA that is ionized to urate will dominate the extracellular plasma and synovial fluid as monosodium urate at pH 7.4 so that the plasma will experience saturation. When UA levels in the body exceed normal limits, UA will build up and cause hyperuricemia (HUA). If ignored, HUA can impair kidney function, such as nephrolithiasis and urate nephropathy, leading to gouty arthritis and cardiovascular disease (Cirillo et al., 2006).

Kidneys play an essential role in filtering blood so that urine is excreted by the body, including maintaining the homeostatic system of UA because the kidneys carry out 70% of UA expenditure. Therefore, HUA and gout are mainly due to a decrease in the relative kidney excretion of UA (Lipkowitz, 2012). When there is an increase in UA levels in the urine, UA will experience saturation and form crystals that eventually form urinary tract stones, which can inhibit and injure the kidneys' tissues and functions and lead to uricosuria.

Previous studies (Angielski, 1992) demonstrated heterogeneity of several enzymes for metabolizing adenosine centered on the rat kidney. The highest activity of ADA, xanthine oxidase, and nucleoside phosphorylase was found in the cytosol in the glomerulus, where the glomerulus is a complex of three different cell types: epithelial, endothelium, and mesangial cells. Increased excessive activity triggers

tissue damage in the kidneys. Several studies have shown that NF $\kappa$ B causes multiple organ damage, such as kidneys, liver, and pancreas (Tugcu et al., 2006). Activated NF $\kappa$ B plays an essential role in expressing pro-inflammatory cytokines such as TNF- $\alpha$  and other mediators involved in the acute inflammatory response and different conditions associated with ROS generation (Arjumand et al., 2011). Several studies have shown this to cause apoptosis and epithelial cells in tubular structures, causing damage to kidney structures and initiating acute kidney poisoning (Abraham, 2003).

HUA is increasing significantly globally because it affects more than 2 billion people (Kramer & Curhan, 2002). In Indonesia, the prevalence of this disease is the second highest, which is 24.7%, and possibly caused mainly by unhealthy eating habits. Allopurinol (ALP) is the most widely used drug because it is considered the most effective in inhibiting the formation of UA by inhibiting the activity of xanthine oxidase (Boudiaf et al., 2010). However, its use can cause serious side effects, such as nephropathy, allergic reactions, and digestive disorders (Feig et al., 2008). The side effects caused by drugs cause some people to choose to use medicinal plants that are considered capable of healing with minimal side effects.

Several medicinal plants have been tested for their effectiveness, such as Indian nettle, rosella (Astuti, 2011), and coffee (Lelyana, 2008). Green tea extract (GTE) (*Camellia sinensis* L.) contains many polyphenol antioxidants, especially flavonoids. The types of flavonoids in GTE are (+)-catechin (C), (-)-epicatechin (EC), (-)-gallo catechin gallate (GCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG) (Jatuworapruk et al., 2014). GTE has strong antioxidant properties by performing several mechanisms, such as anti-lipid peroxidase, free radical scavenger, metal binding, and inhibition of several enzymes, including xanthine oxidase (XOD). Previous studies have shown that Chinese GTE at the right dose can reduce UA content, decrease xanthine oxidase activity (XOD) and URAT1 expression, and increase OAT1 and OAT3 term in the kidneys of mice with HUA (Chen et al., 2015; Fraga et al., 2010).

GTE also contains 8-30 mg of caffeine per cup (240 mL) (Bolignano et al., 2007). Caffeine is a type of alkaloid with the chemical formula 1,3,7-trimethylxanthine (C<sub>8</sub>H<sub>10</sub>O<sub>2</sub>N<sub>4</sub>), is a diuretic. Although several studies have stated that caffeine consumption can trigger excessive UA formation, low intake of caffeine can provide several benefits, such as treating headaches, increasing alertness, and relaxing muscles (Paganini-hill et al., 2007; Zhen et al., 2007). In GTE, caffeine activity is inhibited because of the amino acid L-theanine. To provide a calming effect on the brain (Rogers et al., 2008), L-theanine is also a caffeine antagonist to balance the activity of caffeine in the body

(Kakuda et al., 2000). Thus, in this study, GTE decaffeination did not perform.

## MATERIALS AND METHODS

### Procedures

#### *GTE Leaves Extraction*

Dry GTE leaves (50 g) were crushed by blending and then sifted by a sieve of 80 mesh. GTE powder was brewed using boiled water ( $\pm$  95 °C) with a ratio of 1:10. Then, the result of maceration was cooled to room temperature, filtered, and separated between the filtrate and the dregs. The brewing process was repeated up to 3 times and concentrated using a vacuum rotary evaporator at a temperature of 85 °C and 110 rpm. The GTE's extraction yield was 24.94% (w/w) and kept in a -20 °C freezer.

#### *Acclimatization of Experimental Animals*

The experimental animals used in the study were 24 male albino rats (*Rattus norvegicus*) Wistar strain aged 2-2.5 months with an average body weight of 175-225 g, which were purchased from the provider of laboratory animals in Bandung, Indonesia. All the ethical animal procedures have been approved by UB's Research Commission (Approval Code 690-KEP-UB). The rats were placed in a polyethylene cage filled with wood husks inside with a dimension of 45 x 35 x 20 cm with wire enclosures at the animal laboratory of Biosains of Universitas Brawijaya, Malang, Indonesia. The room temperature was 23 $\pm$ 2 °C. The rats were acclimated for a week before the experiments with a standard feed and water ad libitum.

#### *Induction of High Purine Diet and Drugs Treatment*

Twenty-four rats were divided into six groups, which each group had four rats: (1) negative control group, (2) positive control group, (3) ALP therapy of 5mg/kg of body weight, (4) GTE therapy of 150 mg/kg of body weight, (5) GTE therapy of 300 mg/kg of body weight, and (6) GTE therapy of 600 mg/kg of body weight. Each rat was fed a high purine diet, except the (1) group. Based on a previous study (Rahmawati et al., 2018), we used a high-purine diet consisting of cow's liver, cow's spleen, fried *melinjo* (*Gnemon gnetum*), and fried peanuts blended and dissolved into the water. The filtrate was introduced was 3 mL/rats, twice a day (at 8:00 and 14:00) daily for 60 consecutive days. The UA level was examined every five days. When UA levels were more than 7 mg/dL, rats were ready to be treated.

After UA levels of five groups reached 7 mg/dL, rats in (3) group were administered using 3 mL ALP with a dose of 5 mg/kg of body weight. Rats in (4), (5), and (6) groups were treated with 3 mL of GTE with various doses. All rats were sacrificed for kidney and blood collection on the same day.

### *Blood and Organ Collections*

Blood was drawn from the rats every five days to check the UA levels. Measurements were carried out using a digital tool, "Easy Touch GCU." After calibrated, the test strip was inserted into the detector slit on the device until the screen displayed a "blood drop" image that indicated the device was ready for use. The tail of the rat disinfected with 70% ethanol was massaged, then the tip of the rat's tail was pierced with a sterile blood lancet. The first drop of rat blood was discarded, then the next drip was absorbed into the test strip. Within 20 seconds, the UA level will appear on the device screen in mg/dL. The test was performed on each rat in all groups every day until sacrificing day.

The rats were dislocated in the neck, and the rats were arranged in a supine position ventral above on a surgical board and dissected by splitting the inguinal area. Then both kidneys were taken and washed with 0.9% NaCl. The first kidney was immersed in PBS, while the other was immersed in 4% PFA.

After rats sacrificing, about 2 hours after blood collection, blood was centrifuged at 2000 rpm for 10 min to collect blood serum. Serum was moved into the Eppendorf tube and centrifuged at 1000 rpm for 10 minutes. Serum samples were transferred into a new Eppendorf tube and stored in a -20 °C freezer.

### *Jaffe Test's Measurement of Creatinine Levels*

Serum creatinine levels were determined by enzymatic reactions using the "Point Scientific creatinine kit." 1000 µL of creatinine reagent was taken and put into a microtube, then incubated at 37 °C for 5 minutes. Furthermore, 50 µL of the protein-free filtrate was added, transferred into a cuvette, and the stopwatch was turned on for 60 seconds. The absorbance was measured (A1) using a spectrophotometer with a wavelength of 510 nm, then waited again for 60 seconds, the absorbance was measured again (A2). The absorbance value used is A2-A1.

### *Measurement of MDA Levels*

Half gram of kidney were crushed with quartz sand in a mortar placed on ice gel. Grounded kidneys were added by 1 mL of 0.9% NaCl solution and mixed until homogeneous. The homogenate was put into microtubes, sonicated for 10 minutes, centrifuged at 8000 rpm for 20 minutes, then the supernatant formed was taken carefully using a micropipette. Furthermore, 100 µL of kidney supernatant was taken from each microtube, wrapped in aluminum foil, and added 550 µL of distilled water. Each tube was added with 100 µL of 10% TCA, 100 µL of 1% Na-Thio, and 250 µL of 1M HCl and each tube was homogenized with a vortex. Each tube was incubated in a water bath at 100 °C for 30 minutes and centrifuged at 500 rpm for 10 minutes. Then the centrifuged supernatant was transferred to a new Eppendorf tube. After heating, the supernatant was

cooled at room temperature until a pink complex was formed. The supernatant was then measured for absorbance with a UV-Vis spectrometer at a wavelength of 530 nm. Then the absorbance results were plotted on a curve to calculate the MDA levels.

### **Kidney's Histological Evaluation**

#### *Embedding kidney organs using the Bancroft method*

Kidneys were soaked into a fixative solution of paraformaldehyde, then into 70% ethanol for at least 24 hours, followed by immersion in 80% ethanol for 2 hours. The kidneys were washed with 90% and 95% ethanol, respectively, for 30 minutes. Then, the kidneys were immersed into absolute ethanol three times for 30 minutes, then xylol two times for 30 minutes each in an incubator at 60-63 °C for 30 minutes. The kidneys were immersed in xylol and paraffin three times each. The embedding process was continued by dipping the kidneys into liquid paraffin poured into a container.

#### *Kidney Slide Set Making*

The kidney on the embedding paraffin block was inserted into the microtome block holder, and the cutting surface was aligned with the microtome blade. Cutting begins by adjusting the thickness of the slices above 10 µm. Kidneys were sliced to a size of 5 µm. Slices were taken with a brush and immersed in water at room temperature to reveal possible creases in the slides. The slices were transferred with a brush into warm water at 38-40 °C to straighten the wrinkles then placed on to object-glass. The pieces were dried and placed on a hot plate at 38-40 °C until the slides dry. Furthermore, the slides were stored in an incubator at a temperature of 38-40 °C for 24 hours.

#### *Hematoxylin Eosin Staining Procedures*

Deparaffinization was carried out by putting the slides into xylol three times for 5 minutes each. Then, the rehydration stages were done by putting slides into graded ethanol, starting with absolute ethanol, 95%, 90%, 80%, and 70% ethanol, and distilled water for 5 minutes. The staining stages were carried out by putting the slides into a hematoxylin dye container for 10 minutes. After completed, the slides were washed with running water for 30 minutes, then rinsed with distilled water. The slides were then immersed in eosin alcohol dye for 5 minutes. For the dehydration process, the slides are added to graded ethanol from 80%, 90%, 95%, and absolute ethanol concentrations. The slides are put into xylol two times, dried, and mounted.

### **Statistical Analysis**

The data obtained were analyzed using a data normality test using Shapiro-Wilk statistic and homogeneity to determine the normality of data distribution. Effects of treatment on parameters were analyzed using ANOVA, which was completed by Tukey test with 99%

confidence level to know the difference between treatments. Statistical analysis was performed using Statistical Package for Social Sciences 23.0 software. Results were significant when  $p < 0.01$ .

## RESULTS AND DISCUSSION

### UHPLCMS/MS GTE Analysis

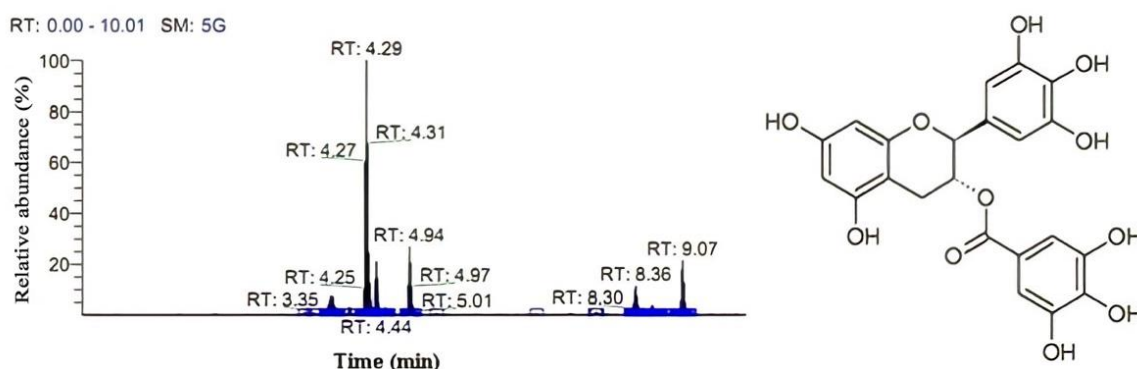
The GTE used was tested qualitatively using UHPLCMS/MS. The result of the qualitative analysis of GTE was presented in **Figure 1**, **Figure 2**, and **Table 1**.

**Table 1.** Parameters in qualitative analysis using UHPLCMS/MS of GTE flavonoid.

RT Peak	Fragment ions ( $m/z$ )	Standard fragment ions ( $m/z$ )	Molecular ions [ $M^+$ ] ( $m/z$ )	Prediction of flavonoid compounds
3.72	136.5-137.5	137	305	(-)-gallo catechin (GC), (-)-epigallocatechin (EGC)
4.29	168.5-169.5	169	457	(-)-epigallo catechin gallate (ECGC), (-)-gallo catechin gallate (GCG)
4.44	204.5-205.5	205	289	(+)-catechin (C), (-)-epicatechin (EC)
4.94	168.5-169.5	169	441	(-)-epicatechin gallate (ECG), (-)-catechin gallate (CG)

Based on **Figure 1**, the GTE was analyzed qualitatively some peaks appeared with its retention time. The highest chromatogram peak from UHPLCMS/MS spectra formed at the retention time of 4.29 min with molecular ions [ $M^+$ ] weight of 457  $m/z$ . Based on the test analysis, the most abundant components in GTE are epigallocatechin gallate

(ECGC) or gallo catechin gallate (GCG) and epicatechin gallate (ECG) or catechin gallate (CG) with the molecular ions [ $M^+$ ] weight of 441  $m/z$  and standard fragment ion of 169  $m/z$ . This peak was identified as epigallo catechin gallate (EGCG) or its epimer, gallo catechin gallate (GCG), with a molecular formula of  $C_{22}H_{18}O_{11}$ .

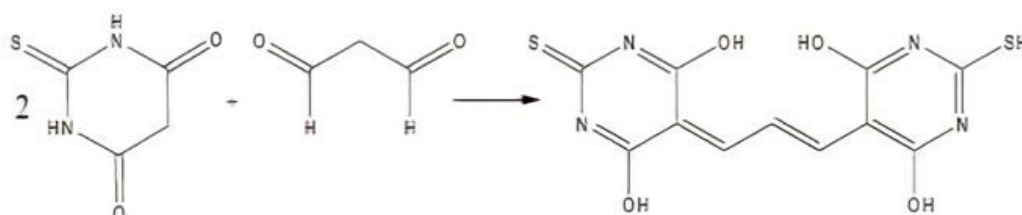


**Figure 1.** UHPLCMS/MS chromatogram of GTE compounds and epigallo catechin gallate (EGCG) or gallo catechin gallate (GCG) molecular structure.

### GTE Effects on MDA Levels in Kidneys

The test was carried out with the thiobarbituric acid test based on the color changes when MDA and TBA reacted. MDA levels in the kidneys indicate how much

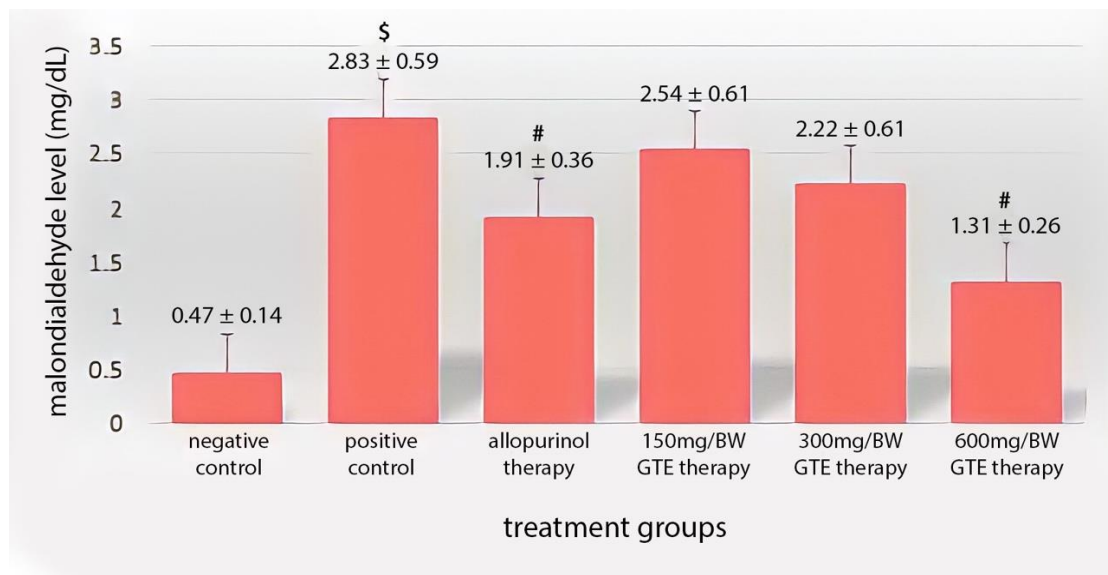
cell damage occurs due to lipid peroxidation due to free radicals formed due to XOD activity. The pink color created was calculated for absorbance at a wavelength of 532 nm.



**Figure 2.** The reaction of MDA and thiobarbituric acid (Nair et al., 1981).

The statistical test results using One Way ANOVA analysis showed that the administration of GTE with three different dosage variations could reduce MDA

levels in the kidneys with a very significant difference between treatments ( $p < 0.05$ ).



**Figure 3.** Graph of MDA Levels in the kidneys. \$ $p < 0.01$  compared to (1). # $p < 0.05$  compared to positive control.

The (1) group had the lowest kidney MDA level of  $0.47 \pm 0.14$  g/dL. This group was a group of rats that were not given any treatment, only given standard food and drink, so they are a group of healthy rats because their kidney MDA levels are still below the normal range, at  $1.04 \pm 0.43$  g/dL.

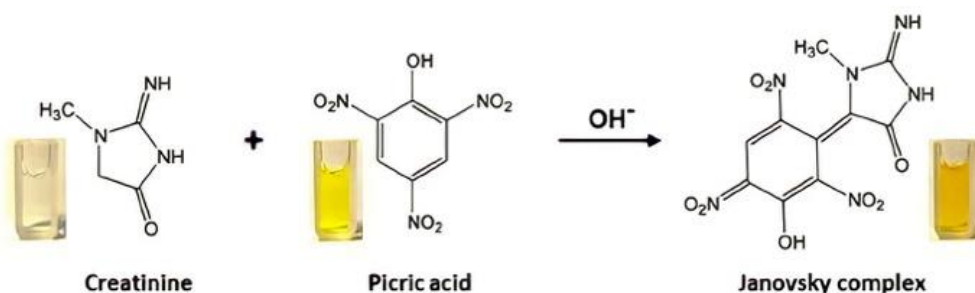
The (2) group was the group that was given a high-purine diet so that the MDA level reached  $2.84 \pm 0.59$  mg/dL. This level is high compared to the (1) group because of the increase of 507.07% compared with the (1) group.

In the (3) group, the levels of MDA formed in the kidneys were  $1.91 \pm 0.36$  g/dL, with a decrease in levels

of 32.67%. In the (4), (5), and (6) groups, the kidney MDA levels were  $2.54 \pm 0.61$  g/dL,  $2.22 \pm 0.61$  g/dL, and  $1.31 \pm 0.26$  g/dL with decreased levels of 10.30 %, 21.56%, and 53.85% respectively.

#### GTE Effects on Creatinine Levels in Serum

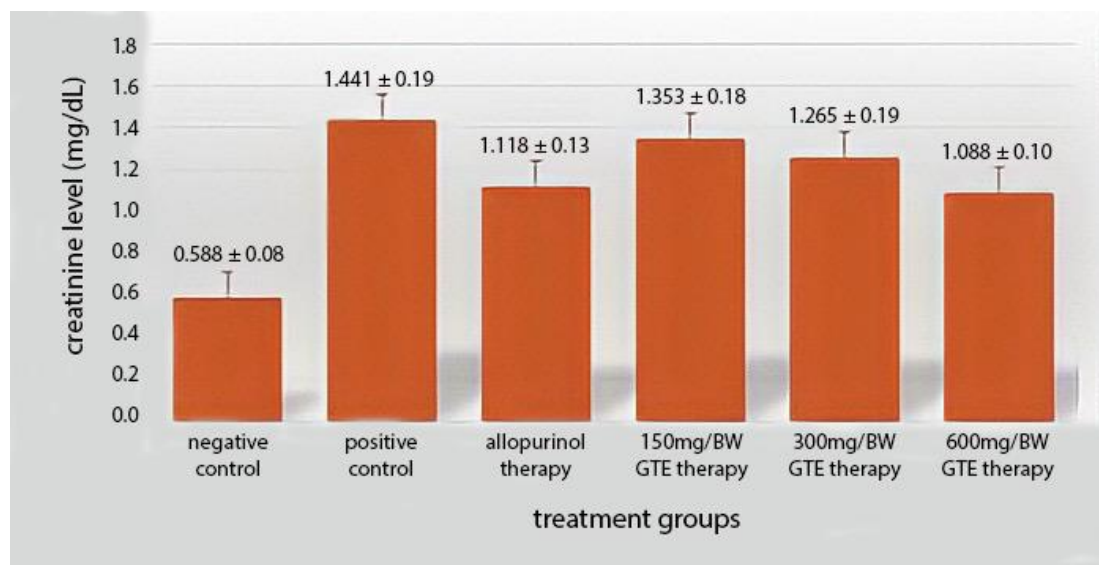
Determination of creatinine levels in blood serum was determined by the Jaffé reaction colorimetric method, with the principle of measuring the absorbance of the picrate reaction in alkaline conditions at a wavelength of 540 nm and creating a complex with a reddish color.



**Figure 4.** The reaction mechanism for the formation of the Janovsky complex (Toora & Rajagopaj, 2002).

The results of the statistical test using one-way analysis of variation (One Way ANOVA) showed that giving GTE with three different dosage variations could

reduce creatinine levels in the kidneys, but not significant ( $p > 0.05$ ).



**Figure 5.** Graph of Creatinine Levels in Serum.

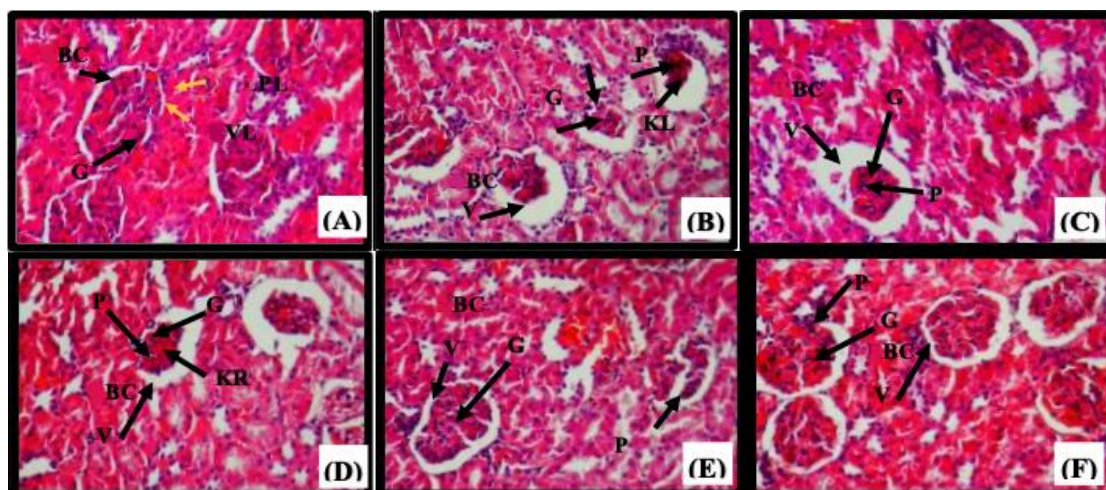
The lowest serum creatinine level was in the (1) group,  $0.59 \pm 0.08$  mg/dL. The positive control group was the group that was given a high-purine diet so that the creatinine level reached  $1.44 \pm 0.19$  mg/dL.

In this study, ALP showed its ability to significantly lower creatinine levels than the (1) groups at  $1.18 \pm 0.13$  mg/dL, with a reduced rate of 22.41%. In this study, there was a significant change and relationship between the decrease in UA levels between positive controls and ALP therapy ( $p = 0.05$ ). In the (4), (5), and (6) group,

the creatinine levels were  $1.35 \pm 0.18$  mg/dL,  $1.26 \pm 0.19$  g/dL, and respectively.  $1.01 \pm 0.10$  g/dL with decreased levels of 6.11%, 12.21%, and 24.50% respectively.

#### GTE Effects on Kidneys Histopathology

Kidneys of rats were examined in histopathology to investigate the level of damage caused by HUA and the improvement that occurred due to ALP therapy and GTE.



**Figure 6.** Kidney histopathology with HE staining (400x magnification); (A) Negative control; (B) Positive control; (C) ALP therapy; (D) GTE therapy of 150 mg/kgBW; (E) GTE therapy of 300 mg/kgBW; (F) GTE therapy of 600 mg/kgBW; (G) Glomerulus; (BC) Bowman's Capsule; (PL) Parietal layer; (VL) Visceral layer; (V) Vacuolization/fat accumulation; (P) Pyknosis; (KR) Karyorrhexis; (KL) Karyolysis

#### Discussion

The structure of EGCG and GCG has eight glycosylate groups that can interact with the active site of the XOD enzyme so that it can inhibit the formation of UA. This fact is in line with previous studies (Chen et al., 2015; Jatuworapruk et al., 2014; Nugraheni et al., 2017) which

reported that GTE could reduce UA levels by inhibiting XOD enzyme activity and modulating urate transporters in the kidneys, thereby increasing the excretion of UA through the kidneys.

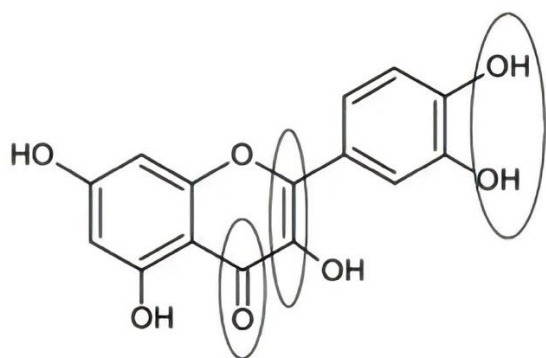
In this study, MDA levels were also determined to determine whether there was an effect of induction of a

high-purine diet on the lipid peroxidation process and the impact of GTE on decreasing MDA levels after a high-purine diet was induced.

The (1) group had the lowest kidney MDA level. This group was used to compare the increase or decrease in kidney MDA levels due to treatment, induction of a high-purine diet, and GTE therapy. The (2) group was the group that was given a high-purine diet so that the MDA level reached 507.07% compared with the negative controls. This high level of MDA is caused by excessive XOD activity and produces ROS in the form of  $H_2O_2$  and  $O_2^{\bullet}$  as a form of innate immunity (Boumerfeg et al., 2012).

Oxidative stress is generally defined as an excess of ROS generation that can damage essential biomolecules such as DNA and lipids massively and cause the formation of chronic diseases such as atherosclerosis, cancer, diabetes, aging, and others, including HUA (Doehner et al., 2016). Excessive XOD activity causes the formation of ROS, which can cause damage to living tissue in the body due to lipid peroxidation. (D.-H. Kang et al., 2002).

In the (3) group, the levels of MDA formed in the kidneys were  $1.91 \pm 0.36$  g/dL, with a decrease in levels of 32.67%. ALP can reduce MDA levels well and prevent kidney damage, consistent with previous studies (Asghar et al., 2007; Chang et al., 2021; Noeman et al., 2011). In the (4), (5), and (6) groups, the kidney MDA levels were decreased 10.30 %, 21.56%, and 53.85%, respectively. This fact is in line with previous studies (Asghar et al., 2007; Noeman et al., 2011), which reported that GTE could reduce MDA levels in the kidneys by inhibiting XOD enzyme activity. The antioxidant capacity of a phenolic antioxidant is directly associated with the ability to donate hydrogen radicals from the phenol group and the presence of unpaired electrons in the aromatic ring.



**Figure 7.** The structure and parts of flavonoids that have potential anti-inflammatory activity (unsaturated C chain, number and position of hydroxyl groups on ring B, carbonyl groups on C-4 carbon, and non-glycosylation) (Lago et al., 2014).

The antioxidant activity of non-glycosylated flavonoids is related to the number and position of the hydroxyl groups present in the molecule. The hydroxyl group in the aromatic core has a low antioxidant

capacity (D. Kang et al., 2005; Soobrattee et al., 2005). This result is in line with previous studies (Cao et al., 2010), (Forester & Lambert, 2011) which reported that flavonoid compounds such as C, EC, and EGC could be ROS absorbers due to 3'-OH and 4'OH hydroxyl groups at ortho positions that increase its antioxidant capacity. The three most important structures are (a) the ortho-dihydroxy catechol at ring B, (b) a double-chain C sigma bond combined with a carbonyl bond at C-4 in ring C, and (c) a hydroxyl group at the C-3 and C-5 position. The EGC structure that fulfills the three requirements above is concluded as the primary agent in the process of reducing ROS activity and reducing lipid peroxidation.

The decrease in kidney MDA levels in ALP therapy was lower than GTE's decrease in MDA levels. This result indicates that ALP can reduce oxidative stress levels by lowering UA levels and improving kidney function. Still, the flavonoids in GTE are more effective in acting as nephroprotection because of their antioxidant activity and hypouricemic effect. Several species of flavonoid antioxidants, especially catechins, are the best ROS absorbers (Boudiaf et al., 2010) by inhibiting the mechanism of ROS generation in tissues and inhibiting the formation of hydroxyl radicals (Lin et al., 2000).

Creatinine was measured as a marker of kidney damage. Creatinine levels above normal limits usually characterize the diagnosis of kidney failure due to low creatinine clearance by the kidneys. The (2) group was the group that was given a high-purine diet so that the creatinine level increased by 145.07% compared to the (1) group. However, these levels are still within the normal range, so this shows that a high-purine diet for 60 days does not cause significant damage to the kidneys.

HUA can induce high blood pressure, increased hydrostatic pressure, and kidney injury (Goicoechea et al., 2010). High UA levels are associated with the onset of kidney-related disease in the late phase. Creatinine is a toxic substance produced when the body usually breaks down creatinine phosphate in muscles and at a constant rate depending on muscle mass. A previous study (D. Kang et al., 2005) found that HUA rats showed higher proteinuria, blood pressure, and serum creatinine levels than the control group treated with ALP as the most effective UA lowering agent. In this study, ALP showed its ability to significantly lower creatinine levels than the (1) group with a reduced rate of 22.41%. By reducing and excreting excess levels of UA in blood serum, ALP can indirectly act as a glomerular hydrostatic pressure-lowering agent and thereby relieve kidney damage. In this study, there was a significant change and relationship between the decrease in UA levels between (2) group and (3) group ( $p = 0.05$ ). Thus, the effect of ALP in reducing the progression of kidney disease may be related to its ability to lower serum UA levels.

In the (4), (5), and (6) groups, the creatinine levels decreased 6.11%, 12.21%, and 24.50%, respectively. Based on statistical analysis using the Post Hoc Tukey test, the decrease in creatinine levels due to ALP therapy and the three doses of GTE therapy did not show a significant average difference. So, it can be concluded that the ability of creatinine-lowering agents from GTE is as good as ALP.

The formation of creatinine begins with the transamination process from arginine to glycine to form glycoxyamine or guanidoacetic acid (GAA). This reaction generally occurs in the kidneys, but the response also appears in the mucosa of the small intestine and pancreas. The creatinine concentration tends to be constant because it is evenly distributed throughout the body. Under normal conditions, creatinine is excreted through the kidneys, but its production increases with age due to decreased muscle mass. With a small molecular weight of about 113 Daltons, creatinine is easily filtered by the glomerulus and is not reabsorbed or affects the urinary rate (Hosten, 1982). The presence of flavonoids that can inhibit the activity of the XOD enzyme can reduce the production of free radicals and reduce the level of oxidative damage (Dahal & Mulukuri, 2015). By decreasing the level of oxidative damage, creatinine can be re-filtered by the glomerulus and excreted adequately by the kidneys.

The (1) group showed normal rat kidney condition, characterized by no vacuolization or fat accumulation, standard glomerular shape, and no signs of necrosis, either pyknosis, karyolysis, or karyorrhexis. This condition is because group A was not given a high-purine diet and was only fed standard water, so there was no significant tissue damage.

Meanwhile, (2) group were given a high-purine diet until the rats experienced HUA. Shriveled glomerulus and blackened nucleus indicate pyknosis caused by damage to membranes, mitochondria, and the Golgi apparatus. The high XOD activity increased the production of ROS and increased the occurrence of lipid peroxidation, which caused tissue damage. Vacuolization also occurs in more significant numbers, caused by cell degeneration due to lipid metabolism disorders that lead to excessive triglyceride accumulation in the cytoplasm with the formation of vacuoles. The cells will experience hypoxia and metabolic disorders. If this happens continuously, it will cause more severe tissue damage, such as karyorrhexis (nuclear rupture and scattering of chromatin fragments scattered around the cell) and karyolysis (the loss of the nucleus due to its inability to be stained), so that the cell pales and disappears. The resulting cell death or necrosis elicits an inflammatory response in living tissues (Asghar et al., 2007).

Kidney histopathology in (3) group showed some improvements. Although vacuolization and pyknosis still happen, the condition of the glomerulus has

improved, and no karyorrhexis and karyolysis has occurred. This condition is due to the ability of ALP to inhibit XOD enzyme activity and inhibit the formation of ROS.

The ultrafiltration of urine causes glomerular damage through the glomerulus, which causes urate crystals not to penetrate the filtration membrane and cause hypertension. The difference in pressure between the glomerular capillary blood pressure and the colloid osmotic pressure in Bowman's capsule can cause glomerular damage. Decreased glomerular filtration is evidenced by increased blood creatinine levels and decreased creatinine clearance so that this condition can be associated with nephrotic syndrome (Dahal & Mulukuri, 2015).

Histopathological appearance of kidneys treated with GTE therapy groups showed some tissue improvement. There was a reduction in vacuolization, pyknosis, and karyorrhexis. This condition shows that green tea can improve the histopathological picture of the kidneys because of its antioxidant activity. It can protect the kidneys from oxidative stress and lipid peroxidation because of its ability to reduce ROS.

Overall, a diet high in purines can cause damage to kidney cell tissue, especially the glomerulus. This fact is shown by creatinine levels that are still in the normal range. However, if a high-purine diet is continued for a long time, glomerular damage can become more severe and lead to further complications of hyperuricemia.

## CONCLUSIONS

It can be concluded from this study that GTE significantly inhibited the activity of the xanthine oxidase enzyme, can lower blood creatinine levels, and reduce malondialdehyde levels in the kidneys. GTE also can improve the kidney histopathological picture of rats with HUA due to a high-purine diet. This study proved that GTE is a promising alternative treatment for HUA. For the following research, it is necessary to conduct further studies on the mechanism of reducing UA with other flavonoid products, such as quercetin, silybin, and luteolin, to compare their effectiveness and determine the effective dose of GTE therapy in lowering UA levels.

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Nugraheni wrote the first draft of the manuscript. Chanif Mahdi provided the critical reading and insightful recommendations of the manuscript. All the authors have read the final manuscript and approved the submission.

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## REFERENCES

- Abraham, E. (2003). Nuclear Factor- $\kappa$ B and Its Role in Sepsis-Associated Organ Failure. *Journal of Infectious Diseases*, 187(Suppl. 2), S364–S369. <https://doi.org/10.1086/374750>
- Angielski, S. (1992). The distribution of enzymes involved in purine metabolism in rat kidney. *Biochimica et Biophysica Acta*, 3, 1–6.
- Arjumand, W., Seth, A., & Sultana, S. (2011). Rutin attenuates cisplatin induced renal inflammation and apoptosis by reducing NF $\kappa$ B, TNF- $\alpha$  and caspase-3 expression in wistar rats. *Food and Chemical Toxicology*, 49(9), 2013–2021. <https://doi.org/10.1016/j.fct.2011.05.012>
- Asghar, M., George, L., & Lokhandwala, M. F. (2007). Exercise decreases oxidative stress and inflammation and restores renal dopamine D1 receptor function in old rats. *American Journal of Physiology. Renal Physiology*, 293(July 2007), F914–F919. <https://doi.org/10.1152/ajprenal.00272.2007>
- Astuti, D. (2011). *Efek Antihiperurisemia Kombinasi Ekstrak Air Kelopak Rosella (Hibiscus sabdariffa L) dan Akar Tanaman Akar Kucing (Acalypha indica L) Pada Tikus Putih Jantan yang Diinduksi Putih Jantan yang Diinduksi Kalium Oksonat*.
- Bolignano, D., Coppolino, G., Barillà, A., Campo, S., Criseo, M., Tripodo, D., & Buemi, M. (2007). Caffeine and the Kidney: What Evidence Right Now? *Journal of Renal Nutrition*, 17(4), 225–234. <https://doi.org/10.1053/j.jrn.2007.02.006>
- Boudiaf, K., Houcher, Z., Widad, S., & Benboubetra, M. (2010). Evaluation of Antioxidant and Anti-Xanthine Oxidoreductase Activities of Nigella sativa Linn seeds ' extracts. *Journal of Applied Biological Sciences*, 4(1), 7–16.
- Boumerfeg, S., Baghiani, A., Djarmouni, M., & Ameni, D. (2012). Inhibitory Activity on Xanthine Oxidase and Antioxidant Properties of Teucrium polium L . Extracts. *Chinese Medicine*, 2012(March), 30–41.
- Cao, H., Pauff, J. M., & Hille, R. (2010). Substrate orientation and catalytic specificity in the action of xanthine oxidase: The sequential hydroxylation of hypoxanthine to uric acid. *Journal of Biological Chemistry*, 285(36), 28044–28053. <https://doi.org/10.1074/jbc.M110.128561>
- Chang, Y. H., Chiang, Y. F., Chen, H. Y., Huang, Y. J., Wang, K. L., Hong, Y. H., Ali, M., Shieh, T. M., & Hsia, S. M. (2021). Anti-inflammatory and anti-hyperuricemic effects of chrysin on a high fructose corn syrup-induced hyperuricemia rat model via the amelioration of urate transporters and inhibition of nlrp3 inflammasome signaling pathway. *Antioxidants*, 10(4), 2005–2008. <https://doi.org/10.3390/antiox10040564>
- Chen, G., Tan, M. L., Li, K. K., Leung, P. C., & Ko, C. H. (2015). Green tea polyphenols decreases uric acid level through xanthine oxidase and renal urate transporters in hyperuricemic mice. *Journal of Ethnopharmacology*, 175, 14–20. <https://doi.org/10.1016/j.jep.2015.08.043>
- Cirillo, P., Sato, W., Reungjui, S., Heinig, M., Gersch, M., Sautin, Y., Nakagawa, T., & Johnson, R. J. (2006). Uric acid, the metabolic syndrome, and renal disease. *Journal of American Society Nephrology*, 17(12 Suppl 3), S165-8. <https://doi.org/10.1681/ASN.2006080909>
- Dahal, A., & Mulukuri, S. (2015). Flavonoids in Kidney Protection. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4(03), 362–382.
- Doehner, W., Jankowska, E. A., Springer, J., Lainscak, M., & Anker, S. D. (2016). Uric acid and xanthine oxidase in heart failure - Emerging data and therapeutic implications. *International Journal of Cardiology*, 213, 15–19. <https://doi.org/10.1016/j.ijcard.2015.08.089>
- Feig, D. I., Soletsky, B., & Johnson, R. J. (2008). Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. *JAMA*, 300(8), 924–932. <https://doi.org/10.1001/jama.300.8.924>
- Forester, S. C., & Lambert, J. D. (2011). The role of antioxidant versus pro-oxidant effects of green tea polyphenols in cancer prevention. *Molecular Nutrition & Food Research*, 55(6), 844–854. <https://doi.org/10.1002/mnfr.201000641>
- Fraga, C. G., Galleano, M., Verstraeten, S. V., & Oteiza, P. I. (2010). Basic biochemical mechanisms behind the health benefits of polyphenols. *Molecular Aspects of Medicine*, 31(6), 435–445. <https://doi.org/10.1016/j.mam.2010.09.006>
- Goicoechea, M., Vinuesa, S. G. De, Verdalles, U., Ruiz-caro, C., Ampuero, J., Rinco, A., & Arroyo, D. (2010). Effect of Allopurinol in Chronic Kidney Disease Progression and Cardiovascular Risk. *Clinical of American Society of Nephrology*, 5, 1388–1393. <https://doi.org/10.2215/CJN.01580210>
- Hosten, A. O. (1982). *BUN and Creatinine*.
- Jatuworapruk, K., Srichairatanakool, S., Ounjaijean, S., Kasitanon, N., Wangkaew, S., & Louthrenoo, W. (2014). Effects of green tea extract on serum uric acid and urate clearance in healthy individuals. *Journal of Clinical Rheumatology*, 20(6), 310–313. <https://doi.org/10.1097/RHU.0000000000000148>
- Kakuda, T., Nozawa, A., Unno, T., Okamura, N., & Okai, O. (2000). Inhibiting Effects of Theanine on Caffeine Stimulation Evaluated by EEG in the Rat. *Bioscience Biotechnology Biochemistry*, 64(2), 287–293.
- Kang, D.-H., Nakagawa, T., Feng, L., Watanabe, S., Han, L., Mazzali, M., Truong, L., Harris, R., & Johnson, R. J. (2002). A Role for Uric Acid in the Progression of Renal Disease. *Journal of the American Society of Nephrology*, 13(12), 2888–2897. <https://doi.org/10.1097/01.ASN.0000034910.58454.FD>
- Kang, D., Park, S., Lee, I., & Johnson, R. J. (2005). Uric Acid – Induced C-Reactive Protein Expression : Implication on Cell Proliferation and Nitric Oxide Production of Human Vascular Cells. *Journal of American of Society Nephrology*, 16(17), 3553–3562. <https://doi.org/10.1681/ASN.2005050572>
- Kramer, H. M., & Curhan, G. (2002). The association between gout and nephrolithiasis: The National Health and Nutrition Examination Survey III, 1988-1994. *American Journal of Kidney Diseases*, 40(1), 37–42. <https://doi.org/10.1053/ajkd.2002.33911>

- Lago, J. H. G., Toledo-Arruda, A. C., Mernak, M., Barrosa, K. H., Martins, M. A., Tibério, I. F. L. C., & Prado, C. M. (2014). Structure-Activity association of flavonoids in lung diseases. *Molecules*, *19*(3), 3570–3595. <https://doi.org/10.3390/molecules19033570>
- Lelyana, R. (2008). *Asam Urat Darah: Studi Eksperimen pada Tikus Rattus norvegicus Galur Wistar*. [http://eprints.undip.ac.id/19270/1/Rosa\\_Lelyana.pdf](http://eprints.undip.ac.id/19270/1/Rosa_Lelyana.pdf)
- Lin, J., Chen, P., Ho, C., & Lin-Shiau, S.-Y. (2000). Inhibition of Xanthine Oxidase and Suppression of Intracellular Reactive Oxygen Species in HL-60 Cells by Theaflavin-3, 3'-di. Inhibition of Xanthine Oxidase and Suppression of Intracellular Reactive Oxygen Species in HL-60 Cells by Theaflavin-3, 3'-di. *Journal of Agricultural and Food Chemistry*, *48*(October), 2736–2743. <https://doi.org/10.1021/jf000066d>
- Lipkowitz, M. S. (2012). Regulation of uric acid excretion by the kidney. *Current Rheumatology Reports*, *14*(2), 179–188. <https://doi.org/10.1007/s11926-012-0240-z>
- Lippi, G., Montagnana, M., Franchini, M., Favaloro, E. J., & Targher, G. (2008). The paradoxical relationship between serum uric acid and cardiovascular disease. *Clinica Chimica Acta*, *392*(1–2), 1–7. <https://doi.org/10.1016/j.cca.2008.02.024>
- Nair, V., Vietti, D. E., & Cooper, C. S. (1981). Degenerative Chemistry of Malondialdehyde, Structure, Stereochemistry, and Kinetics of Formation of Enaminals from Reaction with Amino Acids. *Journal of American Chemistry Society*, *103*(32), 3030–3036.
- Noeman, S. A., Hamooda, H. E., & Baalash, A. A. (2011). Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. *Diabetology & Metabolic Syndrome*, *3*(1), 17. <https://doi.org/10.1186/1758-5996-3-17>
- Nugraheni, P. W., Rahmawati, F., Mahdi, C., & Prasetyawan, S. (2017). Green Tea Extract (*Camellia sinensis* L.) Effects on Uric Acid Levels on Hyperuricemia Rats (*Rattus norvegicus*). *The Journal of Pure and Applied Chemistry*, *6*(3), 246–254.
- Paganini-hill, A., Kawas, C. H., & Corrada, M. M. (2007). Non-alcoholic beverage and caffeine consumption and mortality: The Leisure World Cohort Study ☆. *Preventive Medicine*, *44*, 305–310. <https://doi.org/10.1016/j.ypmed.2006.12.011>
- Rahmawati, F., Nugraheni, P. W., Mahdi, C., Srihardyastutie, A., & Prasetyawan, S. (2018). Optimization Of Elevating Blood Uric Acid Levels With High Purine Diet. *The Journal of Pure and Applied Chemistry Research*, *7*(1), 19–25. <https://doi.org/10.21776/ub.jpacr.2018.007.01.357>
- Rogers, P. J., Smith, J. E., Heatherley, S. V., & Pleydell-Pearce, C. W. (2008). Time for tea: mood, blood pressure and cognitive performance effects of caffeine and theanine administered alone and together. *Psychopharmacology*, *195*, 569–577. <https://doi.org/10.1007/s00213-007-0938-1>
- Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I., & Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, *579*(1–2), 200–213. <https://doi.org/10.1016/j.mrfmmm.2005.03.023>
- Toora, B., & Rajagopaj, G. (2002). Measurement of creatinine by Jaffe's reaction - Determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum. *Indian Journal of Experimental Biology*, *40*(1), 352–354.
- Tugcu, V., Ozbek, E., Tasci, A. L. I. I., Kemahli, E., & Bas, M. (2006). Selective nuclear factor k-B inhibitors, pyrrolidium dithiocarbamate and sulfasalazine, prevent the nephrotoxicity induced by gentamicin. *Journal Compilation BJU International*, *98*, 680–686. <https://doi.org/10.1111/j.1464-410X.2006.06321.x>
- Zhen, M., Wang, Q., Huang, X., Cao, L., Chen, X., Sun, K., Liu, Y., Li, W., & Zhang, L. (2007). Green tea polyphenol epigallocatechin-3-gallate inhibits oxidative damage and preventive effects on carbon tetrachloride – induced hepatic fibrosis. *Journal of Nutritional Biochemistry*, *18*, 795–805. <https://doi.org/10.1016/j.jnutbio.2006.12.016>