JURNAL NATURAL
Vol. 24, (3) 2024
DOI 10.24815/jn.v24i3.29599
pISSN 1411-8513
eISSN 2541-4062
ORIGINAL RESEARCH

Green tea (*Camellia sinensis L.)* potential to liver of hyperuricemia rats (*Rattus norvegicus*) fed with high purine diet

PUTRANTY WIDHA NUGRAHENI[1]*, CHANIF MAHDI[2], FITRIA RAHMAWATI[2] , RIZKI NUR ANALITA[4]

[1]Department of Environmental Engineering, University of Tanjungpura
[2]Department of Chemistry, University of Brawijaya
[4]Department of Chemistry Education, University of Lambung Mangkurat

*Corresponding Author:
putranty@teknik.untan.ac.id

Received: 17 December 2022
Revised: 11 June 2024
Accepted: 14 October 2024

Abstract. Uric acid is produced in tissues containing xanthine oxidases, like the small intestine and liver, as involved in the purine metabolism of adenine and guanine. High uric acid levels cause damage to cell membranes due to the lipid peroxidase chain reaction. Foods containing high purines will activate the xanthine oxidase (XOD), causing an increase in free radicals. Free radicals attack polyunsaturated fatty acids (PUFA) by forming lipid peroxides. PUFA molecules contain multiple double bonds, which make them susceptible to oxidation by reactive oxygen species (ROS), such as free radicals. PUFA will be broken into simple compounds such as pentane, ethane, and aldehydes, leading to malondialdehyde (MDA) formation. High MDA levels illustrate the process of cell membrane oxidation leading to cell membrane damage. Hyperuricemia is often treated using synthetic drugs such as allopurinol; however, it can cause side effects. Some people will choose medicinal plants with minimal side effects. Green tea (*Camellia sinensis* L.) contains many polyphenol antioxidants, especially flavonoids, which also have a strong antioxidant effect by exerting multiple mechanisms such as inhibiting several enzymes, including antiphospholipid peroxidase, free radical scavengers, metal bonds, and xanthine oxidase (XOD). Statistical analysis results proved that green tea with a dose of 600 mg/kg BW could decrease the level of MDA in the liver by 84.87% (p <0.01) and reduce the activity of XOD by 35.36% (p <0.01). Taken together, green tea can improve liver histopathology.

Keywords: green tea extract, hyperuricemia, liver histopathology, liver malondialdehyde, xanthine oxidase

INTRODUCTION

Uric acid (UA) is a weak acid with a p*K*a of 5.8 [1], [2], and the reference value for normal blood uric acid levels in men is 3.5 – 7.0 mg/dL, while in women is 2.4 – 5.7 mg/dL. UA is the resulting product of purine breakdown. It acts as an antioxidant, but excessive amounts in the blood will act as a pro-oxidant [3] and cause crystallization, leading to gout [4]. Under the microscope, the urate crystals resemble tiny needles that are sharp, white, and have a foul odor [5]. The formation of uric acid in the blood can also increase, caused by external factors, especially foods and drinks that stimulate the formation of uric acid. When the UA build-up in the body is interrupted, the excretion process is interrupted, which will cause a build-up of uric acid in the kidneys and joints, leading to impaired kidney function [6].

Xanthine oxidase (XOD) plays a role in the formation of forming radical oxygen species (ROS). Adenine and xanthine are produced from the breakdown of ATP and xanthine dehydrogenase (XDH)) turns into XOD. As a result, during the reperfusion process, XOD utilizes molecular oxygen from respiration as a substrate rather than NAD^+, producing superoxide anion (O_2^-) free radicals. [7]. Lack of ATP in muscle and formation of ADP causes an increase in the production of hypoxanthine and xanthine, then stimulates an increase in xanthine oxidase activity. Consequently, the production of ROS with highly reactive oxygen species such as hydroxyl radicals (OH^-•), hydrogen peroxide (H_2O_2•), superoxide radicals (O_2•), and other radical species [8], [9] also increases. The enzyme oxidoreductase catalyzes the reaction of uric acid synthesis from hypoxanthine and xanthine in two convertible forms that use molecular oxygen as an

electron acceptor and form superoxide anions and other reactive oxygen products, causing oxidative stress [10].

In general, the treatment of hyperuricemia is often done using synthetic drugs such as allopurinol; however, it can trigger side effects [11]. Some people prefer medicinal plants that are thought capable of healing with few side effects concerning the side effects of pharmaceuticals. Numerous medicinal plants have undergone examination to determine their effectiveness in reducing uric acid levels, such as cat's whiskers [12], rosella [13], and coffee [14]. One of the plants that was thought to reduce uric acid levels is green tea (*Camellia sinensis* L.). Numerous polyphenol antioxidants, particularly flavonoids, are present in green tea. Green tea contains a variety of flavonoids, including (+)-catechin (C), (–)-epicatechin (EC), (–)-gallocatechinalatine (GCG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin (ECG), and (–)-epigallocatechin gallate (EGCG) [15]. Green tea possesses powerful antioxidant capabilities via multiple pathways, including anti-lipid peroxidase, free radical scavenger, metal binding, and inhibition of various enzymes, including xanthine oxidase (XOD) [16]. Several researchers have conducted numerous studies on uric acid on a laboratory scale to determine the potential effect of catechins from green tea on uric acid levels by using animal models[17], [18], [6], [19]. Catechins also inhibit the formation of lipid peroxidation at the initiation stage by acting as a scavenger against reactive oxygen free radicals (O_2^-) and hydroxyl radicals (OH^-) to reduce MDA levels and XOD activity [20], [21].

Besides flavonoids, green tea contains caffeine, an alkaloid compound [22]. Caffeine can have several advantages, including relieving headaches, boosting alertness, and relaxing muscles. Numerous studies have reported that caffeine might generate excess uric acid [23], [24]. L-theanine, found in green tea, acts as a caffeine antagonist, thereby reducing the stimulatory effects of caffeine and maintaining a balanced activity in the body [25]; furthermore, the amino acid L-theanine calms the brain [26]. Therefore, no decaffeination on green tea leaves was carried out in this study.

Green tea has not been optimally used as herbal medicine. Therefore, it is necessary to test the potential of catechins from green tea for hyperuricemia therapy. In this study, the test was carried out using rats as experimental animals to examine xanthine oxidase activity in rat livers, MDA levels in rat livers, and rat liver histopathology. The organ studied in this study was the liver because it contains the xanthine oxidase enzyme, where the process of synthesizing purines into uric acid occurs. This research will provide more information about green tea's potential to treat hyperuricemia.

METHODOLOGY

Green Tea Leaves Extraction

The dried green tea leaves were ground in a blender, sieved using an 80-mesh filter, and then macerated with 95 ºC distilled water at a ratio of 1:10 for 30 minutes. The filter paper was used to separate the filtrate from the biological material. The filtrate was stored, and the maceration process was repeated up to 3 times using the same method. Furthermore, all the filtrate obtained was then combined and subsequently concentrated with a rotary evaporator vacuum at a temperature of 85 ºC with a speed of 110 rpm.

Phytochemical Test

The physicochemical tests of the aqueous green tea leaf extract were determined and carried out in this study, including qualitative and quantitative, including flavonoids and polyphenols. The qualitative tests done were flavonoid and polyphenol to test and identify the compounds in green tea to ensure that green tea contains flavonoid compounds. The quantitative test used UHPLC (Ultra-High-Performance Liquid Chromatography) and MS (Mass Spectrophotometry)**.**

UHPLC-MS/MS Analysis

As much as 0.1 g of green tea leaf extract was dissolved in pure methanol up to 1 mL. The extract was filtered with a PTFE (polytetrafluoroethylene polymer) membrane filter with a gaze size of 0.2 microns. The filtrate formed was put into a 1.5 mL vial. The flavonol standard test used UHPLC and MS/MS (Ultra-High-Performance Liquid Chromatography and Mass Spectrophotometry). The UHPLC used was ACCELLA type 1250 made by Thermo Scientific, which consisted of a degasser vacuum, a quaternary pump, and a thermostatic autosampler controlled by a personal computer with the X-Calibur 2.1 program. The mobile phase used was 0.1% formic acid in methanol (A) and 0.1% formic acid in methanol (D) with a flow rate of 300 L/minute with the following settings: 0-1 minutes 5% D; 1-5 minutes 50% D; 5-6 minutes 90% D; 6-7 minutes 90% D. A total of 2µL of the sample was injected in the LC and the temperature was controlled at 30 ºC in the column and 16 ºC in the autosampler compartment.

The MS/MS tool used is the Triple Q (quadrupole) mass spectrophotometer TSQ QUANTUM ACCESS MAX made by Thermo Finnigan with an ionization source from ESI (Electrospray Ionization). ESI ionization conditions: spray voltage 3kV, evaporation temperature 250 ºC, capillary temperature 300 ºC, nitrogen pressure as sheath gas 40 psi, and argon pressure as aux gas 10 psi. The device is controlled by TSQ Tune software in positive operation mode. Determination of the presence of catechins and their derivatives is carried out using the SRM (Selected Reaction Monitoring) method.

Acclimatization of Animal Model

Rats (*Rattus norvegicus*) aged 2 – 2.5 months old rats with a body weight of 175 – 225 grams were previously adapted for one week in the Animal Biosciences Laboratory, Universitas Brawijaya Malang, Indonesia, before being used for experiments. Rats were placed in polyethylene cages with 45 x 35 x 20 cm dimensions with wood husks at 23±2 ºC in a predetermined humidity and given standard feed and water ad libitum. After adaptation, a pre-examination was carried out by checking uric acid levels using the test kit *Easy Touch GCU*®. The Universitas Brawijaya Research Ethics

Commission has approved all animal treatment procedures with the approval code 690-KEP-UB.

Optimization of Hyperuricemia Animal Model Fed by High Purine Diet

After seven days of acclimatization, thirty rats were separated into six groups: (a) the negative control, (b) the positive control, (c) the allopurinol therapy at 5 mg/kg body weight, (d) the green tea therapy (I) at 150 mg/kg body weight, (e) the green tea therapy (II) at 300 mg/kg body weight, and (f) the green tea therapy (III) at 600 mg/kg body weight. Except for the negative group, each rat was fed a high-purine diet. A high purine diet consisting of fried melinjo (*Gnemon gnetum*), fried peanuts, and bovine liver was prepared based on the research done by Rahmawati [25]. For 60 consecutive days, the high purine feed was given at 3 mL/rat twice daily (at 8:00 A.M. and 2:00 P.M.). The uric acid value was assessed every five days using the test kit Easy Touch GCU®. The rat was prepared for treatment when the UA level reached 7 mg/dL or higher.

Animal Model Treatment

When the uric acid level reached 7 mg/dL, rats in groups (c), (d), (e), and (f) were administered treatments according to what has been described in the previous sub-chapter. At 8.00 A.M., the rats were given a diet high in purines, and at 2:00 P.M., they were treated with green tea extract at various doses at 150 mg/kg body weight, 300 mg/kg body weight, and 600 mg/kg body weight, for 14 days. At the end of the study, all rats were sacrificed for total uric acid levels analysis and the liver for analysis of XOD (xanthine oxidase), MDA (malondialdehyde acid), and liver histopathology [28].

Measurement of XOD Levels

1 g of the liver was weighed and cut into pieces. Then, the liver was homogenized with 50 mM cold phosphate buffer (pH 7.5) in a ratio of 1:5 and centrifuged at 3000 rpm for 10 minutes to produce a supernatant fraction. The supernatant was separated and centrifuged again at 4000 rpm for 120 min. This supernatant will be used to test the activity of xanthine oxidase [29].

Xanthine oxidase activity was tested by monitoring the formation of uric acid using spectrophotometric methods. Test tube containing 1.9 mL of 50 mM of potassium phosphate buffer solution in pH 7.5 added with 1 mL of 2.1 mM of xanthine and 0.1 ml of xanthine oxidase supernatant then incubated at 20 ºC for 45 minutes. After the mixture had been set, 1 mL of 0.58 M HCl was added immediately to stop the reaction. To determine the quantity of unreacted xanthine still present in the test sample, the mixture's absorbance was examined using a UV spectrophotometer at 293 nm [30].

Measurement of Liver's MDA Levels

As much as 0.5 g of the liver was weighed, ground with the quartz sand in a mortar, then added 1 mL of NaCl-Fis 0.9% and homogenized. The homogenate was put in the microtube and sonicated for 10 minutes and centrifuged at 8000 rpm at 25 ºC for 10 minutes. 100 µL of liver supernatant obtained from the homogenate from each rat was taken, then added with 550 µL of distilled water. The mixture was added with 100 µL of 10% TCA (trichloroacetic acid), 250 µL of 1 N HCl, and 100 µL of Na-Thiobarbituric acid. At each addition of reagents, the mixed solution is homogenized with the help of a vortex. The mixture was then incubated for 30 minutes in a water bath at 100 ºC, cooled at room temperature, then centrifuged at 500 rpm for 10 minutes. The absorbance of the sample was measured at a maximum wavelength of 530 nm and plotted on a standard curve that had been made to calculate the sample concentration.

Liver's Histopathological Evaluation

The first step of the liver's histopathological evaluation is embedding the liver by soaking it in a 4% PFA solution. The dehydration process was carried out on the slides by being put in graded ethanol, starting from 70% ethanol for 24 hours, 80% ethanol for 2 hours, 90% ethanol for 30 minutes, 95% ethanol for 30 minutes, and absolute ethanol three times for 30 minutes in different bottles. The dehydrated liver was immersed in the xylol solution 2 times for 30 minutes each, then soaked again in the xylol solution in an incubator at 60 – 63 ºC for 30 minutes. The liver was dipped in liquid paraffin 3 times, and then the block was embedded in liquid paraffin in a container and allowed to solidify.

The next step is to position the liver tissue parallel to the microtome blade by placing it into the previously inserted paraffin block on the microtome block holder. Adjusting the stability of the slices above 10 m to accelerate the 5 m-thick cutting plane is the first step in the cutting process. The cuts were removed with a brush and placed in 38–40 °C warm water to remove any potential wrinkles in the preparations. Using an object-glass, the results of adequately stretched slices are obtained. The chosen parts were dried and placed on a hot plate at 38–40 °C until the slide was completely dry. The slides were then incubated at 38–40 °C for 24 hours.

Staining the liver begins with the deparaffination stage, where the slides are added to xylol in 1-3 steps for 5 minutes 3 times each. The rehydration process was carried out on the slides by adding them to graded ethanol, starting with absolute ethanol for 5 minutes 3 times, 95% ethanol, 90% ethanol, 80% ethanol, and 70% ethanol each for 5 minutes. Afterward, they soaked in distilled water for 5 minutes. Furthermore, the slides were stained with hematoxylin staining for 10 minutes until the best results were obtained, then washed in running water for 30 minutes, rinsed with distilled water, and put in eosin alcohol dye for 5 minutes. Then, the slides were immersed in distilled water to remove excess eosin. Then, the dehydration step was carried out, and the slides were added to graded ethanol from 80% to 90% and absolute ethanol 3 times. Then, it was cleared by inserting the slides in xylol for 5 minutes 2 times, air-dried, and mounted with entellan, and the slides were covered with a cover glass [31].

Table 1. Peak results and interpretation of flavonoid compound types contained in aqueous green tea leave extract

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	(–)-epigallocatechin (EGC) (–)-gallocatechin (GC)
4.29	168.5-169.5	169	457	(–)-gallocatechin gallate (GCG) (–)-epigallocatechingallate (ECGC)
4.44	204.5-205.5	205	289	(–)-epicatechin (EC) (+)-catechin (C)
4.94	168.5-169.5	169	441	(–)-catechingallate (CG) (–)-epicatechingallate (ECG)

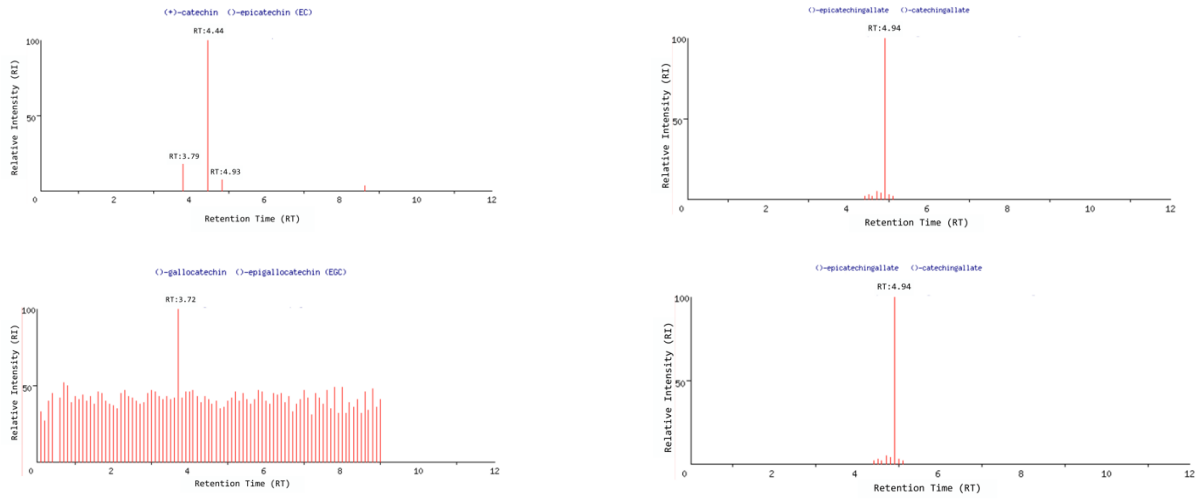

Figure 1. Qualitative chromatogram of green tea extract with UHPLCMS/MS

RESULTS AND DISCUSSION

Phytochemical Analysis

The results of green tea extraction were carried out by brewing with boiling water to obtain the maximum amount of extract, and concentration was carried out using a rotary evaporator to produce an extract in the form of a black-green paste of 12.47 grams with a yield of 24.94%. The extract analysis revealed the existence of specific phytochemicals, including alkaloids, flavonoids, and catecholic tannin. This was evident through distinct color changes observed in the solution, turning it yellow, white, and green-black.

Analysis of Green Tea Leaves Extract by UP-HPLC/MS

Green tea leaves contain polyphenolic compounds that are effective antioxidants for the body, especially flavonoids (Table 1). One of the flavonoids in green tea is flavonol, which contains several compounds such as epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), epicatechin gallate (ECG), epicatechin (EC), and catechin (C) [32], [33]. In this study, a qualitative analysis was carried out on green tea using LC/MS to determine whether there were flavonol compounds and their derivatives, as mentioned above. UP-HPLC/MS combines chromatographic separation techniques with compound identification based on molecular weight so that almost all compounds in the sample can be identified with just one injection. However, UP-HPLC/MS cannot be used to measure quantitatively, and measurements can be made if the specific type of compound to be analyzed is known and must be completed with a pure standard solution. The UP-HPLC/MS spectra results show the number of peaks corresponding to several flavonoids.

The qualitative analysis of green tea using LC-MS showed that green tea leaves contain compounds C, EC, GC, EGC, EGCG, GCG, ECG, and CG in Figure 1. The most abundant compounds contained in green tea leaves are Epigallocatechin gallate (EGCG) or Gallocatechingallate (GCG), with a parent mass of 457 m/z and product mass of 169 m/z and Epicatechin gallate (ECG) or Catechingallate (CG) with parent mass m/z 441 m/z and product mass 169 m/z.

Green Tea Leaves Extract Potential to XOD Levels

The xanthine oxidase (XOD) converts purines into hypoxanthine, xanthine, and uric acid. The number of purines entering the metabolism will cause the enzyme activity of the xanthine oxidase and increase uric acid levels in the blood. Xanthine oxidase can be found in liver and muscle cells [34], [35], but excessive xanthine oxidase activity in the blood indicates impaired liver function.

The results showed that therapy with green tea extract could reduce the activity of the XOD enzyme (Fig. 2). Based on the statistical analysis results, green tea with a dose of 150 mg/kg BW can reduce XOD activity but not significantly ($p<0.05$). Meanwhile, green tea extract therapy with doses of 300 and 600 mg/kg BW and allopurinol 5 mg/kg BW could reduce XOD activity by

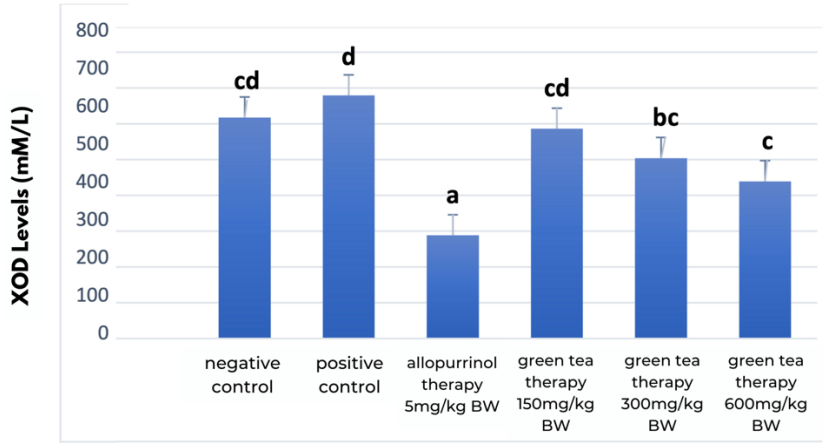

Figure 2. Graph of average XOD activity in rat liver. a < 0.05 compared to ab and c < 0.01 compared to a. The notation a, b, c, and d indicate significant differences between groups (a) the negative control, (b) the positive control, (c) the allopurinol therapy at 5 mg/kg body weight, (d) the green tea therapy (I) at 150 mg/kg body weight, (e) the green tea therapy (II) at 300 mg/kg body weight, and (f) the green tea therapy (III) at 600 mg/kg body weight.

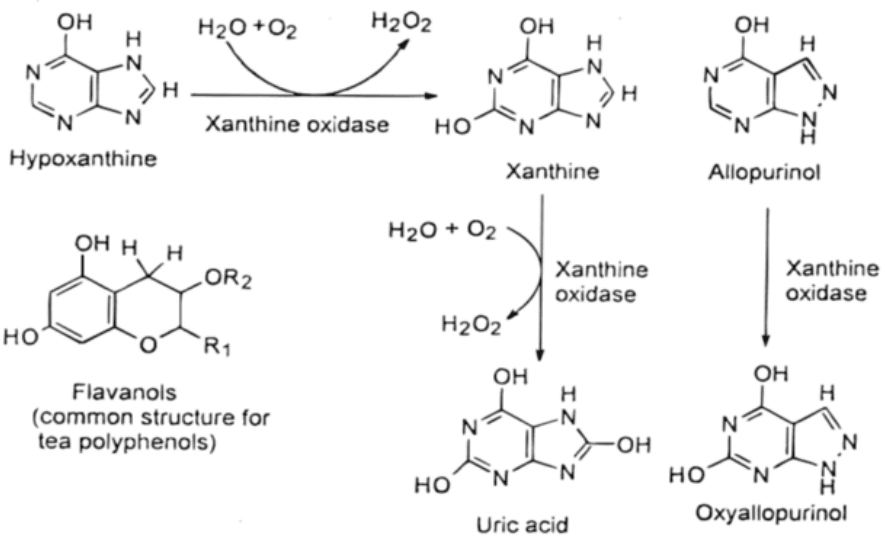

Figure 3. The structure of hypoxanthine, xanthine, uric acid, allopurinol, and flavonols. Xanthine and hypoxanthine are substrates for XOD, and allopurinol is a substrate analog [10]

showing a significant difference between treatments (p<0.01).

Based on the measurement results, it is known that the XOD activity of the negative control group showed the second-highest value of the positive control at 617.06 ± 10.62 mM/L minutes and experienced an increase in XOD activity of 10.01%. In the positive group, those with a high-purine diet had the highest XOD activity; the value was 678.82 ± 34.45. The value of XOD activity in the positive control group was used to determine whether an increase or decrease occurred because of the treatment given.

The results of measuring XOD activity in the control group with green tea extract therapy with several variations in doses of 150 (I), 300 (II), and 600 (III) mg/kg BW were 585.89 ± 31.47; 503.88 ± 14.25; and 438.81 ± 46.55 mM/L respectively, and decreased XOD activity by 13.69 %, 25.77%, and 35.36 % respectively. These results indicate that green tea extract therapy with a dose of 600 mg/kg BW was the ablest to reduce XOD activity compared to therapy (I) and (II). Meanwhile, the allopurinol control drug yielded an average XOD activity of 288.145 ± 99.845 with a decreased XOD activity of 57.55%. Based on Figure 3, allopurinol can significantly reduce XOD activity because the structure of allopurinol is like hypoxanthine that binds to the active site of the xanthine oxidase enzyme as a competitive inhibitor should be provided with hypoxanthine. Meanwhile, therapy with green tea extract has not been able to significantly reduce XOD activity because the active compounds contained in green tea, flavonoids, have a slightly different form from hypoxanthine and can reduce XOD activity by binding the hydroxyl group to the active site of XOD; thus, flavonoids in green tea

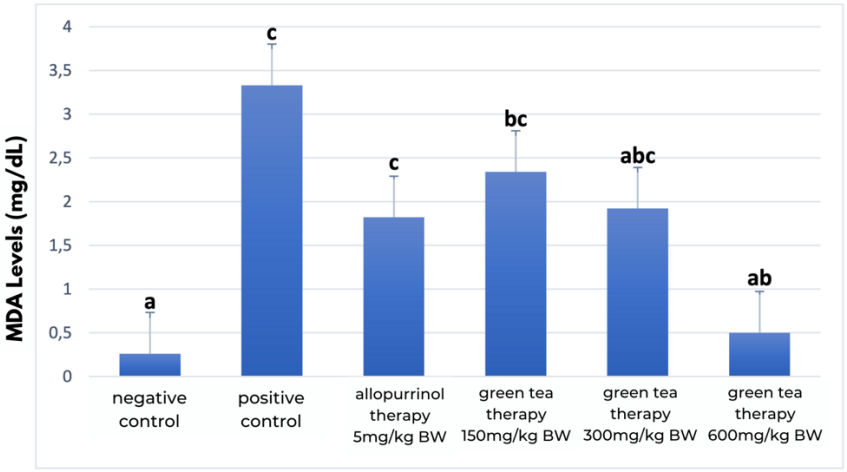

Figure 4. Graph of average MDA levels in rat liver. c < 0.01 compared to a. b < 0.05 compared to c and ab < 0.01 compared to c. The notation a, b, c, and d indicate significant differences between groups (a) the negative control, (b) the positive control, (c) the allopurinol therapy at 5 mg/kg body weight, (d) the green tea therapy (I) at 150 mg/kg body weight, (e) the green tea therapy (II) at 300 mg/kg body weight, and (f) the green tea therapy (III) at 600 mg/kg body weight.

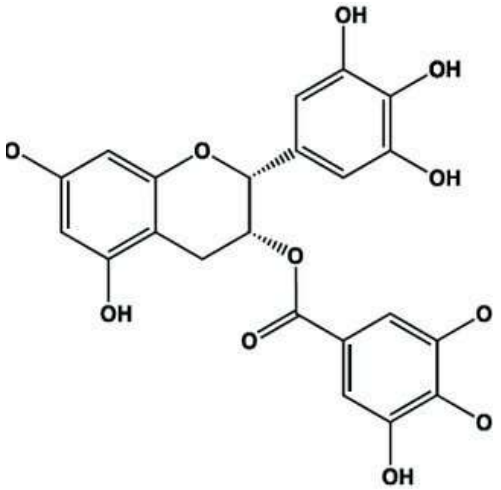

Figure 5. The structure and constituents of (–)–Epigallocatechin gallate (EGCG), one of the flavonols of the flavonoid family found in green tea, with anti-inflammatory properties (unsaturated C chain, number, and position of hydroxyl groups on ring B, carbonyl groups on C4 carbon, and non-glycosylation).

extract is not as significant as allopurinol in reducing XOD activity.

XOD enzyme inhibition test in vivo showed that green tea extract has the potential as a xanthine oxidase inhibitor and is productive as an antigout. The potential for this inhibitor is due to the content of secondary metabolites in each green tea extract flavonoids, which have the potential to inhibit the work of the xanthine oxidase enzyme. The relationship between the structure of flavonoids and their activity as a xanthine oxidase inhibitor is due to the presence of hydroxyl groups (OH groups) at C5 and C7, and the double bond between C2 and C3 in ring B will result in the position of ring B being co-planar to ring A, making it easier to interact. With xanthine oxidase, double bonds in flavonoids allow them to conduct addition reactions (oxidation by xanthine oxidase) [36]. The ability of flavonoids to inhibit XOD activity occurs through competitive inhibition mechanisms and interactions with enzymes in group sides [10].

Aqueous Green Tea Leaves Extract Potential to MDA Levels of Liver

High levels of purines that enter the body will increase the activity of the xanthine oxidase enzyme in converting purines into hypoxanthine, xanthine, and uric acid using oxygen as a catalyst. This reaction produces a byproduct of superoxide anion radicals ($O_2\bullet^-$) which can cause damage to cell membranes through the formation of lipid peroxidation and formation of malondialdehyde (MDA). High MDA levels indicate an oxidation process or cell membrane damage due to free radicals.

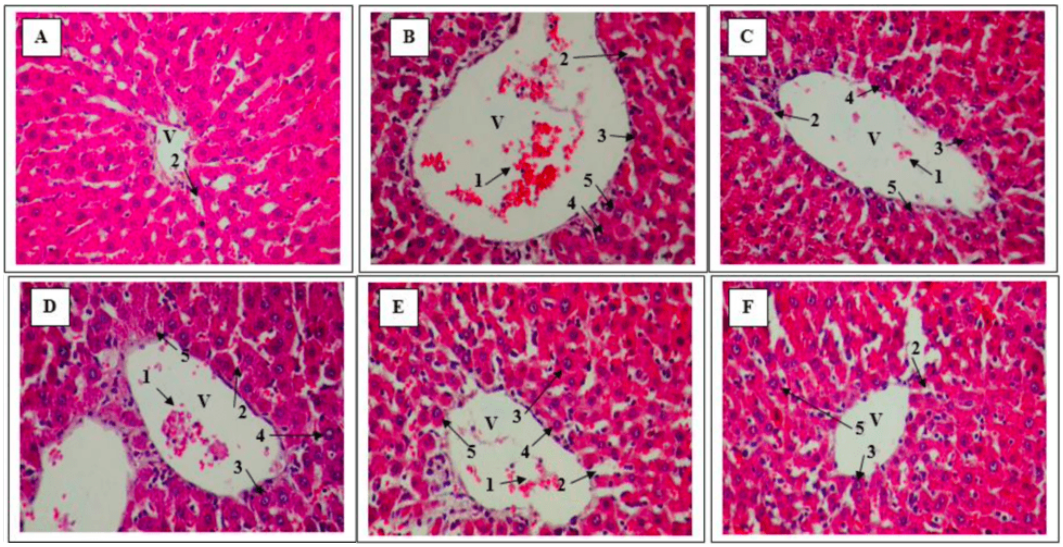

Figure 3. Histopathology image of rat's liver slides at 400x magnification (A) Negative control, (B) Positive control, (C) Drug therapy 5 mg/kg BW, (D) Therapy 1 150 mg/kg BW, (E) Therapy II 300 mg/kg BW, (F) Therapy III 600 mg/kg BW (V) Central vein, (1) Erythrocyte dam, (2) Sinusoid, (3) Pyknosis, (4) Karyolysis and (5) Karyorrhexis

MDA is a compound resulting from lipid peroxidation, which is toxic due to oxidative stress events in the body. MDA levels in the liver indicate cellular damage due to lipid peroxidation by free radicals or ROS. The higher the MDA level, the higher the free radical level. MDA is formed only by fatty acids with three or more double bonds.

The MDA levels in the negative control group had the lowest average of 0.260 g/mL (Fig. 4). This value indicates the expected value of the average MDA level in normal or healthy rats so that it can be used to determine whether there is an increase or decrease in MDA levels due to the influence of treatment. Meanwhile, the average MDA level of the hyperuricemia rats group had the highest value compared to the intermediate MDA level of the negative group, which was 3.330 g/mL. MDA levels in the positive group increased by 1,280.77%. The average MDA levels in the group of rats treated with aqueous green tea leaves extract for 2 consecutive weeks were 2.339 g/mL in therapy (I), 1.922 g/mL in therapy (II), and 0.504 g/mL in therapy (III), meanwhile for allopurinol therapy was 1.815 g/mL. The average MDA levels in treated rats decreased compared to the positive control group, with 29.78%, 42.30%, and 84.87%, respectively. The average liver MDA levels in therapy (III) were the best compared to the other therapies, which was 84.87%. Allopurinol can reduce oxidative stress levels and reduce MDA levels in rat liver. However, the content of flavonoid compounds in green tea is more effective in lowering uric acid levels due to its antioxidant activity.

Under normal conditions, oxidase will convert hypoxanthine and xanthine into uric acid using oxygen as a catalyst. This reaction produces a byproduct of superoxide anion ($O_2\bullet^-$). Furthermore, the body's antioxidant system, the superoxide dismutase (SOD) enzyme, converts it into H_2O_2, and the catalase enzyme converts it again into H_2O. Nevertheless, a high purine diet will cause xanthine oxidase activity to increase 20 times compared to customary conditions, so the formation of superoxide radicals increases. Superoxide radicals are highly reactive free radicals that can cause damage to cell membranes through the formation of lipid peroxidation [37].

Carbon radicals are produced when hydrogen ions in cell membranes' side chains (PUFA) combine with free radicals to create lipid peroxidase. The oxidation of carbon radicals will generate peroxyl radicals. In addition, the peroxyl radicals will bind H^+ ions on the neighboring PUFA side chain, resulting in lipid peroxidation. This process is a chain reaction because lipid peroxidation attracts H^+ ions to other PUFA side chains until the PUFA chain is broken into other compounds, such as hydrocarbons 5-hydroxynonenal and aldehyde compounds, resulting in the creation of MDA. High levels of MDA indicate oxidation or cell membrane damage caused by free radicals [38].

The measurement of MDA levels in the treatment group with green tea extract in various doses can reduce MDA levels due to flavonoid groups in green tea. One of the active compounds of green tea is catechin, which acts as an antioxidant that can act as a potent antioxidant and anti-inflammatory. The ability of catechins to scavenge free radicals is 100 times more effective than vitamin E and 25 times more effective than vitamin C [39]. This finding is consistent with prior research [40], [41], which reported that flavonoid compounds such as C, EC, and EGC might be radical oxygen species reducers due to the presence of 3'-OH and 4'OH hydroxyl groups at the ortho position, which boosts their antioxidant activity that calm down before malondialdehyde activity. The three most significant structures are (a) the ortho-dihydroxy

catechol in ring B, (b) a double-chain C sigma bond paired with a carbonyl bond at C4 in ring C, and (3) a hydroxyl group at positions C3 and C5. It is determined that the EGC structure that satisfies the three characteristics mentioned earlier is the crucial agent in lowering ROS activity and lipid peroxidation.

Aqueous Green Tea Leaves Extract Potential for Liver Histopathology

This study also used liver histopathological parameters using Hematoxylin-Eosin (HE) staining. Histopathological images were used to determine the level of liver tissue damage in hyperuricemia rats. Observations using a microscope with a magnification (400x).

Figure 6 shows the liver histopathology of rats starting from the negative control group, the positive control group, the allopurinol group at a dose of 5 mg/kg BW, therapy (I) at a dose of 150 mg/kg BW, therapy (II) at a dose of 300 mg/kg BW and therapy (III) at a dose of 600 mg/kg BW. The histopathological observations of the liver in negative control rats showed normal histology, which was characterized by the absence of erythrocyte damming in the central vein, and the cells did not undergo necrosis.

In positive control, cells experienced necrosis and damming of erythrocytes in the central vein due to blockage of the hepatic vein. If this blockage lasts long enough, liver cells appear lost due to stress and disturbances in carrying nutrients. The blood that flows from the periphery of the liver lobules to the central vein has lost nutrients when it arrives in the middle of the lobules, which leads to necrosis [42]. The presence of pyknosis, karyorrhexis, and karyolysis characterizes necrosis. The shrinkage of the cell nucleus depicts pyknosis, the disintegration of the nucleus characterizes karyorrhexis, and the fragments of chromatin are scattered in the cell. Karyolysis is characterized by the cell nucleus losing the ability to be stained or appearing pale, vaguely hollow, and disappearing.

The presence of necrosis will cause an inflammatory response in living tissue. The occurrence of cells experiencing necrosis in the positive control was under the results of the average MDA level of the positive control mice, which was 3.30 ± 0.971 mg/dL because of the continuous administration of a high-purine diet that causes an increase in free radicals in the body due to activity disrupting of the xanthine oxidase. These free radicals bind to O_2 in the body to form peroxyl radicals that absorb hydrogen atoms from unsaturated lipid molecules, resulting in a prolonged reaction that produces peroxides such as peroxynitrite. This peroxide is lipophilic and causes lipid peroxidation in the membrane and further increases the number of ions in the body, such as Na^+, K^+, Fe^{2+}, and $Cu^{2+,}$ that cause necrosis of liver cells [28], [43].

A histopathological picture of the liver in therapy (I) showed that the cells were still undergoing necrosis, but the damming of erythrocytes in the central vein was slightly reduced. This result is consistent with MDA levels of 2.339 ± 0.249 mg/dL, which decreased somewhat compared to positive controls. In therapy (II), the necrotic cells began to decline, and there was a slight damming of erythrocytes in the central vein, with the MDA levels being 1.922 ± 1.028 mg/dL. Meanwhile, fewer necrotic cells and no erythrocytes were damming in the central vein in the therapy (III) group.

The allopurinol drug group with a dose of 5 mg/kg BW showed similar results to therapy group II, but the erythrocyte damming in the central vein was less in the control drug. Histopathological observations on the liver of rats showed that green tea extract therapy was able to repair tissue in the therapy (III); this was also supported by the results of MDA levels in the liver, which decreased by 84.87% because the antioxidant activity content of flavonoid compounds in green tea that can repair liver tissue.

CONCLUSION

Based on the research findings, it can be inferred that green tea extract therapy can inhibit xanthine oxidase (XOD) activity and malondialdehyde (MDA) levels in rats fed a high-purine diet and enhance their liver histopathology. Flavonoids, prominent secondary plant metabolites, have been documented to exhibit diverse pharmacological properties, such as inhibiting xanthine oxidase and anti-inflammatory effects. The presence of a C2–C3 double bond (C-ring) and hydroxyl groups at positions C3′, C4′ (B-ring), C5, and C7 (A-ring) are vital structural prerequisites for a flavonoid molecule to exhibit both anti-inflammatory and XOD activities inhibitor simultaneously. The microscopic examination of liver histopathology in rats administered with an extract of green tea leaves revealed that the optimal improvement in liver histopathological structure was observed at a dose of 600 mg/kgBW. This dose exhibited a significant enhancement in normal cells and binucleic cells. The presence of catechin effectively protects the liver against fatty liver induced by a high purine diet. This was evidenced by an increase in the number of normal cells and a decrease in necrotic cells. In addition to the liver, the enzyme xanthine oxidase is also present in the small intestine, which calls for assessing the enzyme's activity in this organ.

Furthermore, it is necessary to conduct histological examinations of the joints to determine the presence of urate crystals. Further extensive research is required to enhance the optimal utilization of green tea extract as a herbal plant. Additionally, conducting studies to identify the levels of individual phytochemicals in neem leaves is crucial to identify compounds that actively contribute to improving liver cell tissues.

ACKNOWLEDGMENT

We thank the Indonesian Ministry of Finance for supporting this project through the Indonesia Education Endowment Fund (LPDP *Kementerian Keuangan Republik Indonesia*).

REFERENCE

[1] Maiuolo, J.; Oppedisano, F.; Gratteri, S.; Muscoli, C.; Mollace, V. 2015. Regulation of uric acid metabolism and excretion. *Int. J. Cardiol.* **213** 8-14. DOI: 10.1016/j.ijcard.2015.08.109.

[2] Gliozzi, M.; Malara, N.; Muscoli, S.; Mollace, V. 2016. The treatment of hyperuricemia. *Int. J. Cardiol*. **213**. DOI: 10.1016/j.ijcard.2015.08.087.

[3] Forester, S. C.; Lambert, J. D. 2011. The role of antioxidant versus pro-oxidant effects of green tea polyphenols in cancer prevention. *Mol. Nutr. Food Res.* **55**(6) 844-854. DOI: 10.1002/mnfr.201000641.

[4] Martinon, F.; Petrilli, V.; Mayor, A.; Tardivel, A.; Tschopp, J.; Pétrilli, V. 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature.* **440**(7081) 237-241. DOI: 10.1038/nature04516.

[5] Mittal, M.; Patra, S.; Saxena, S.; Roy, A.; Yadav, T.; Vedant, D. 2022. Gout in Primary Hyperparathyroidism, Connecting Crystals to the Minerals. *J. Endocr. Soc.* **6**(4). DOI: 10.1210/jendso/bvac018.

[6] Nugraheni, P. W.; Mahdi, C. 2022. Kidney Evaluation in Hyperuricemia Rats Treated with Green Tea Leaves (Camellia sinensis L.) Extract. *Biol. Med. Nat. Prod. Chem.* **11**(1) 17-26. DOI: 10.14421/biomedich.2022.111.17-26.

[7] Kelley, E. E.; Khoo, N. K. H.; Hundley, N. J.; Malik, U. Z.; Freeman, B. A.; Tarpey, M. M. 2010. Hydrogen peroxide is the major oxidant product of xanthine oxidase. *Free Radic. Biol. Med.* **48**(4) 493-498. DOI: 10.1016/j.freeradbiomed.2009.11.012.

[8] Gois, P. H. F. et al. 2016. Allopurinol attenuates rhabdomyolysis-associated acute kidney injury: Renal and muscular protection. *Free Radic. Biol. Med.* **101** 176-189. DOI: 10.1016/j.freeradbiomed.2016.10.012.

[9] 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. *J. Agric. Food Chem.* **48** 2736-2743. DOI: 10.1021/jf000066d.

[10] Nagao, A.; Seki, M.; Kobayashi, H. 1999. Inhibition of xanthine oxidase by flavonoids. *Biosci. Biotechnol. Biochem.* **63**(10) 1787-1790. DOI: 10.1271/bbb1961.49.2173.

[11] Schlesinger, N.; Radvanski, D. C.; Young, T. C.; McCoy, J. V.; Eisenstein, R.; Moore, D. F. 2015. Diagnosis and Treatment of Acute Gout at a University Hospital Emergency Department. *Open Rheumatol. J.* **9**(1) 21-26. DOI: 10.2174/1874312901409010021.

[12] Tabana, Y. M. et al. 2016. Cat's whiskers (Orthosiphon stamineus) tea modulates arthritis pathogenesis via the angiogenesis and inflammatory cascade. *BMC Complement. Altern. Med.* **16**(1). DOI: 10.1186/s12906-016-1467-4.

[13] 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. Skripsi. Depok: Universitas Indonesia.

[14] Choi, H. K.; Curhan, G. 2007. Coffee, Tea, and Caffeine Consumption and Serum Uric Acid Level: The Third National Health and. *Arthritis Rheum*. **57**(5) 816-821. DOI: 10.1002/art.22762.

[15] Bruno, R. S.; Dugan, C. E.; Smyth, J. A.; DiNatale, D. A.; Koo, S. I. 2008. Green tea extract protects leptin-deficient, spontaneously obese mice from hepatic steatosis and injury. *J. Nutr.* **138** 323-331.

[16] Hofmann, E. et al. 2016. Hydroxylated chalcones with dual properties: Xanthine oxidase inhibitors and radical scavengers. *Bioorg. Med. Chem.* **24**(4) 578-587. DOI: 10.1016/j.bmc.2015.12.024.

[17] Kanji, T.; Gandhi, M.; Clase, C. M.; Yang, R. 2015. Urate lowering therapy to improve renal outcomes in patients with chronic kidney disease: systematic review and meta-analysis. *BMC Nephrol.* **16**(1). DOI: 10.1186/s12882-015-0047-z.

[18] Preitner, F. et al. 2013. Urate-induced acute renal failure and chronic inflammation in liver-specific Glut9 knockout mice. *Am. J. Physiol. Renal Physiol.* **305**(5) F786-795. DOI: 10.1152/ajprenal.00083.2013.

[19] Ohishi, T.; Goto, S.; Monira, P.; Isemura, M.; Nakamura, Y. 2016. Anti-inflammatory Action of Green Tea. Antiinflamm. *Antiallergy Agents Med. Chem.* **15**(2) 74-90. DOI: 10.2174/1871523015666160915154443.

[20] Chen, L. et al. 2007. Relationship between hyperuricemia and metabolic syndrome. *J. Zhejiang Univ. Sci. B.* **8**(8) 593-598. DOI: 10.1631/jzus.2007.B0593.

[21] Pribadi, F. W.; Ernawati, D. A. 2010. Efek Catechin terhadap Kadar Asam Urat, C-Reaktive Protein (CRP) dan Malondialdehid Darah Tikus Putih (Rattus norvegicus) Hiperurisemia. *Mandala Health*. **4**(1) 39-46.

[22] Prasanth, M.; Sivamaruthi, B.; Chaiyasut, C.; Tencomnao, T. 2019. A Review of the Role of Green Tea (Camellia sinensis) in Antiphotoaging, Stress Resistance, Neuroprotection, and Autophagy. *Nutrients.* **11**(2) 474. DOI: 10.3390/nu11020474.

[23] Gopal, J.; Muthu, M.; Paul, D.; Kim, D. H.; Chun, S. 2016. Bactericidal activity of green tea extracts: The importance of catechin containing nano particles. *Sci. Rep.* **6** 19710 DOI: 10.1038/srep19710.

[24] Luk, H. Y. et al. 2020. Impacts of green tea on joint and skeletal muscle health: Prospects of translational nutrition. *Antioxidants.* **9**(11) 1-28. DOI: 10.3390/antiox9111050.

[25] Kahathuduwa, C. N. et al. 2020. Effects of l-theanine-caffeine combination on sustained attention and inhibitory control among children with ADHD: a proof-of-concept neuroimaging RCT. *Sci. Rep.* **10**(1). DOI: 10.1038/s41598-020-70037-7.

[26] Baba, Y.; Inagaki, S.; Nakagawa, S.; Kaneko, T.; Kobayashi, M.; Takihara, T. 2021. Effects of l-Theanine on Cognitive Function in Middle-Aged and Older Subjects: A Randomized Placebo-Controlled Study. *J. Med. Food.* **24**(4) 333-341. DOI: 10.1089/jmf.2020.4803.

[27] 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. Diabetol. *Metab. Syndr.* **3**(1) 17. DOI: 10.1186/1758-5996-3-17.

[28] Afsar, T.; Razak, S.; Almajwal, A. 2019. Effect of Acacia hydaspica R. Parker extract on lipid peroxidation, antioxidant status, liver function test and histopathology in doxorubicin treated rats. *Lipids Health Dis.* **18**(1) 126. DOI: 10.1186/s12944-019-1051-2.

[29] Rashidinejad, A.; Birch, E. J.; Everett, D. W. 2016. Green tea catechins suppress xanthine oxidase activity in dairy products: An improved HPLC analysis. *J. Food Compos. Anal.* **48** 120-127. DOI: 10.1016/j.jfca.2016.03.001.

[30] Lin, S.; Zhang, G.; Liao, Y.; Pan, J. 2015. Inhibition of chrysin on xanthine oxidase activity and its inhibition mechanism. *Int. J. Biol. Macromol.* **81** 274-282. DOI: 10.1016/j.ijbiomac.2015.08.017.

[31] Alwahaibi, N.; Alkhatri, A.; Kumar, J. 2015. Hematoxylin and eosin stain shows a high sensitivity but sub-optimal specificity in demonstrating iron pigment in liver biopsies. *Int. J. Appl. Basic Med. Res.* **5**(3) 169. DOI: 10.4103/2229-516X.165365.

[32] Rashidinejad, A.; Birch, E. J.; Everett, D. W. 2016. Green tea catechins suppress xanthine oxidase activity in dairy products: An improved HPLC analysis. *J. Food Compos. Anal.* **48** 120-127. DOI: 10.1016/j.jfca.2016.03.001.

[33] Susanti, E.; Ciptati; Ratnawati, R.; Aulanni'am; Rudijanto, A. 2015. Qualitative analysis of catechins from green tea GMB-4 clone using HPLC and LC-MS/MS. Asian Pac. *J. Trop. Biomed.* **5**(12) 1046-1050. DOI: 10.1016/j.apjtb.2015.09.013.

[34] Zhao, X.; Zhu, J. X.; Mo, S. F.; Pan, Y.; Kong, L. D. 2006. Effects of cassia oil on serum and hepatic uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J. Ethnopharmacol.* **103**(3) 357-365. DOI: 10.1016/j.jep.2005.08.040.

[35] Zhu, J. X.; Wang, Y.; Kong, L. D.; Yang, C.; Zhang, X. 2004. Effects of Biota orientalis extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J. Ethnopharmacol.* **93**(1) 133-140. DOI: 10.1016/j.jep.2004.03.037.

[36] Dahal, A.; Mulukuri, S. 2015. Flavonoids in Kidney Protection. *World J. Pharm. Pharm. Sci.* **4**(03) 362-382.

[37] Rubbo, H.; Radi, R.; Prodanov, E. 1991. Substrate inhibition of xanthine oxidase and its influence on superoxide radical production. *Biochim. Biophys. Acta.* **1074**(3) 386-391. DOI: 10.1016/0304-4165(91)90089-Y.

[38] Traverso, N. et al. 2004. Malondialdehyde, a Lipoperoxidation-Derived Aldehyde, Can Bring About Secondary Oxidative Damage To Proteins. *J. Gerontol.* **59**(9) 890-895.

[39] Cos, P. et al. 1998. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers***. J. Nat. Prod.*** **61**(1) 71-76. DOI: 10.1021/np970237h.

[40] Hayashi, T.; Sawa, K.; Kawasaki, M.; Arisawa, M.; Shimizu, M.; Morita, N. 1988. Inhibition of Cow's Milk Xanthine Oxidase by Flavonoids. *J. Nat. Prod.* **51**(2) 345-348.

[41] Diwan, V.; Brown, L.; Gobe, G. C. 2017. The flavonoid rutin improves kidney and heart structure and function in an adenine-induced rat model of chronic kidney disease. *J. Funct. Foods.* **33** 85-93. DOI: 10.1016/j.jff.2017.03.012.

[42] 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. Diabetol. *Metab. Syndr.* **3**(1). DOI: 10.1186/1758-5996-3-17.

[43] Ayala, A.; Muñoz, M. F.; Argüelles, S. 2014. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid. Med. Cell. Longev*. **14** 1-31. DOI: 10.1155/2014/360438.

How to cite this article:
Nugraheni, P.W., Mahdi, C., Rahmawati, F., Analita, R.N. (2024). Green Tea (*Camellia sinensis* L.) potential to liver of hyperuricemia rats (Rattus norvegicus) fed with high purine diet. Jurnal Natural, 24(3), 146-155.