IJPSR (2021), Volume 12, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 July 2020; received in revised form, 01 July 2021; accepted, 02 July 2021; published 01 August 2021

BREAD FRUIT LEAF EXTRACT COMPOSITION AND ITS EFFECT ON OXALATE AND LEUKOCYTE PROFILES IN HYPEROXALURIC RATS INDUCED BY ETHYLENE GLYCOL

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Keywords:

GC-MS, Breadfruit, Nepherolithiasis, Oxalate, Leukocyte

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ABSTRACT: Nephrolithiasis or kidney stone disease is a disease characterized by the formation of crystals in the kidney. It is a disease that is found in many countries with high rates of recurrence and treatment costs. This study analyzes the effect of *Breadfruit* leaf extract on oxalate levels of CaOx stones deposited in the kidney and leukocyte differentiation in hyper oxaluric rats induced ethylene glycol. Experimental research with a -post-test with control design using 25 rats, which randomly divides into five groups, namely Normal, Ethylene Glycol, extract doses of 100, 200, and 400 mg/kg BW groups. The rat's induced ethylene glycol 0.75% v/v for 28 days to initiate hyperoxaluria and the formation of CaOx stones. The treatment groups were given extracts from days 15 to 28. The data show tht the induction of ethylene glycol increases the levels of kidney oxalate (CaOx). Giving *Breadfruit* leaf extract 400 mg/kg BW can significantly reduce kidney oxalate (CaOx) levels and influence the total level of leukocytes. Leukocyte differentiation. The Breadfruit leaf extract prevents the formation of calcium oxalate kidney stones and decreases inflammatory markers too.

INTRODUCTION: Nephrolithiasis is a condition marked by the presence of renal calculi. The worldwide prevalence, incidence, and composition of calculi vary and have changed in the 7% to 13% in North America, 5%-9% in Europe, and last several decades, with prevalence ranging from 1%-5% in Asia [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(8).4525-34

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(8).4525-34

The cost of health care and drug costs is very burdensome to the state budget because it relies more on curative rather than preventive illness Recurrence is a problem; the United States (US) spent 2.1 billion dollars on stone disease in 2000.

This is in comparison to the 1.83 billion dollars the US spent on the same disease five years prior. In 2030, the projected amount of spending on nephrolithiasis in the US is set to be 4.1 billion dollars [2]. These numbers only include direct costs. ROS overproduction or decreased antioxidants lead to oxidative stress, inflammation and injury, and are involved in stone comorbidity. All major chronic inflammation markers are detectable in

stone patient urine [3]. The high oxalate, calcium oxalate and calcium phosphate crystals provoked renal cell reactive oxygen species mediated inflammatory responses. Calcium oxalate crystals induce renin up-regulation and angiotensin II generation. Nonphagocytic NADPH oxidase leads to ROS production mediated by protein kinase C. The P-38 MAPK/JNK transduction pathway is turned on. Transcriptional and growth factors, and generated secondary mediators become involved. MCP-1, NF-kβ and OPN production is increased and macrophages infiltrate the renal interstitium around the crystal.

Thiazide-type (TZ) diuretics are commonly used as a treatment for Ca stones. The use of a TZ-type drug (hydrochlorothiazide, chlorthalidone, or indapamide) can significantly decrease the recurrence of stones compared with placebo over three years. Thiazide diuretic use is associated with lower serum magnesium levels and an increased risk of hypomagnesaemia.[6] High use of hydrochlorothiazide (HCTz) (≥50,000 mg) was associated for BCC (basal cell carcinoma) and SCC (squamous cell carcinoma), and found clear dose-response relationships between HCTz use and both BCC and SCC [5, 6, 7]. ESWL shows good results in the treatment of kidney stones up to 2.0 cm and is an alternative to ureteroscopy in the management of ureteral calculi, Minor complications may occur following ESWL, which generally respond well to clinical interventions. The relationship between ESWL and hypertension/diabetes is not well established, [8] and can increase stones hardness due to the conversion of CaOx to CaP.

The formation of CaOx stones in the kidney causes injury to epithelial cells, which are manifested by pain, high temperature, redness, and inflammation. Injury of tubular epithelial cells triggers several pro-inflammatory cytokines toward damaged tubular cells. The presence of pathogenesis causes directing leukocytes to the site of injury to perform the function to return to a balanced state [8].

Breadfruit or *Artocarpus altilis*, is an Indonesian native plant traditionally used for the treatment of various disease including inflammatory-related diseases. Studies of the anti-inflammatory activity of Aaltilis leaf extract in mice, showed that the COX-2 expression level in hind legs was also significantly reduced in dose-dependent treatment of *A. altilis* leaf extract. This indicates that the leaf extract of *A. altilis* has the potential to be further developed as an anti-inflammatory agent or as a source of lead compounds that act as anti-inflammatory. A compound is said to have antioxidant activity when there is a capture of free radicals through the hydrogen atom donor from compound group [10]. Our research has shown that the *Breadfruit* leaf extract has antioxidant effects, decreases creatinine And BUN levels, and improves kidney tissue damage in hyper oxaluricrats [11]. This study aims to analyze the effect of the *breadfruit* leaf extract on oxalate levels and leukocyte differentiation in urolithic rats induced by ethylene glycol.

MATERIALS AND METHODS:

Chemicals and Reagents: Quercetin (standard), ethanol 96% (Pharmaceutical grade), N-hexane (E-Merck), ethyleneglycol (E-Merck, sodium chloride (Otsuka), Na oxalic (E-Merck)p.a, KMnO4(E-Merck)p.a.

Plant Material: *Breadfruit* leaf (Moraceae) available during August from the local cultivating field area of Ungaran, Semarang Regency, Central Java, Indonesia. Authenticate Plant material at Ecology and Biosystematic Laboratory of Biology Department, Faculty of Mathematics and Natural Sciences, Diponegoro University, Semarang. Indonesia. The results of plant authentication are 1b, 2b, 3b, 4b, 6b, 7b, 9b, 10b, 11b, 12b, 13b, 14a, 15a, Group 8: Plants with single and scattered leaves, 109b, 119b, 120a, 121b, 124a, Family 38: Moraceae Genus 2. Artocarpus Species: Artocarpus communis *Breadfruit*. The leaves are cleaned and simplified, turning dried leaves into powder using a mechanical method, and filtering the leaf powder in a 40 # sieve, and storing it in an airtight container,

Preparation of *Breadfruit* Leaf Extracts: Accurately weigh 500 g of breadfruit leaf powder taken in a stainless steel container and mixed with 5000 ml (1:1020) of alcohol 70%. Then the mixture was tightly closed and left for three days protected from light, while repeatedly stirring after two days, then filtered. Masticate is placed on a porcelain cup while the residue is added with enough liquid to leave it for one day while stirring occasionally.

The extract obtained was filtered using a flannel cloth. Then all the juice results were combined and evaporated using a rotary evaporator at 500 °C until a thick *breadfruit* leaf extract was obtained [12]. Purify the crude extract with 100 mlof n-hexane, then shaken and allowed to stand until there are two separate layers. The ethanol layer is taken and concentrated with a rotary evaporator using a temperature of 50 °C so that the alcohol fraction is obtained.

The alcoholic extract is used for treatment with doses 8; 16, and 32 mg/ml, the volume of administration of 2.5 ml orally.

Preparation of Ethylene Glycol 0,75% % V/V: As much as 0.75 ml of EG pipetted put into a measuring gourd, and added distilled water to the limit of 100 ml, the volume of administration of 2.5 ml orally.

GC-MS Analysis: The extract (0.10 µl) was injected to GC-MS analysis in Shimadzu QP 2010, with the injection temperature being 220 °C. The flow rate of Helium gas as a carrier gas is 1 ml/min; meanwhile, the interface temperature of GC-MS was 250 °C.

The compound was identified based on the mass spectral database. The GC-MS analysis of *breadfruit* leaf extract was conducted at the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University Yogyakarta, Indonesia.

Total Flavonoid Content (TFC) Assay: Extract is made for residues with the aim that the solvent evaporates. To the residue, add 5 ml of 0.1 M ammonium chloride then incubate (40 min) at room temperature absorbance measurement at 415 nm Shimadzu UV mini 1240. Quercetin standard plot was used to evaluate TFC and expressed as mg QE / g DW from *breadfruit* ethanol extract.

Oxalate Level Analysis in the Kidney: A part of the kidney is taken and weighed, then cut into small pieces and put into a glass beaker; saline solution is added and heat for 30 min.

Filter to separate the kidneys from dissolved oxalate. The oxalate levels were determined by the Permanganometry method using standard Sodium oxalate.

Leukocyte Analysis: Blood collection through the orbital plexus in the eye using micro hematocrit after the rat has been anesthetized. The flowing blood is collected in a microtube and centrifuged. Plasma was analyzed with an automatic blood analyzer. The absolute value of leukocyte differentiation obtained was taken randomly from each group compared to the absolute value of the literature

Animals: Experimental research with post-test design with the control group, 25 male Rattus norvegicus Wistar strain, bodyweight 180-200 g, age ± 3 months, are divided into 5 groups were taken for the study. Animals were obtained from the small animal breeding station.

TABLE 1: EXPERIMENTAL DESIGN OF OXALATE AND LEUKOCYTE DIFFERENTIATION STUDIES

Groups	Treatments (days)	
	1-14	15-28
Normal	Water	water
EG	EG 0,75% v/v	EG 0,75% v/v
BE1	EG 0,75% v/v	EG+ BE 100 mg/kgBW
BE2	EG 0,75% v/v	EG+ BE 200 mg/kgBW
BE3	EG 0,75% v/v	EG+ BE 400 mg/kgBW

EG: Ethylene glycol; BE: *Breadfruit* extract

Ethical Clearance: The feasibility of research ethics was carried out in the Research Ethics Commission of the Faculty of Veterinary Medicine, Gadjah Mada University, based on Ethical Clearance No. 0140 / EC-FKH / Eks / 2019 declared to meet ethical requirements to be implemented.

Statistical Analysis: Values were represented as mean ± S.E.M, Statistical analysis using one-way ANOVA and post hoc test.

RESULTS:

Gc-Ms Analysis of *Breadfruit* Leaf Extract Content: The GC-MS image extract shows the presence of 3 peaks associated with the compound.

The detected compounds are presented in **Table 1**, while the chromatogram and structure are presented respectively in **Fig. 1** and **2.**

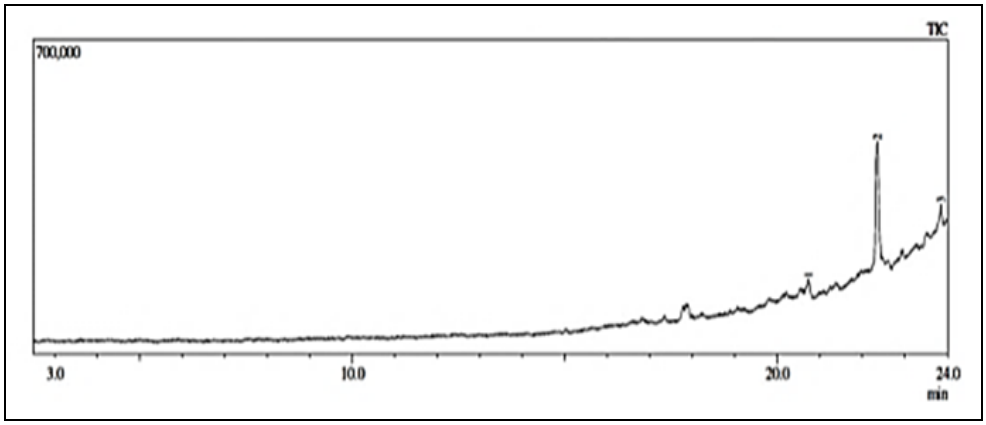
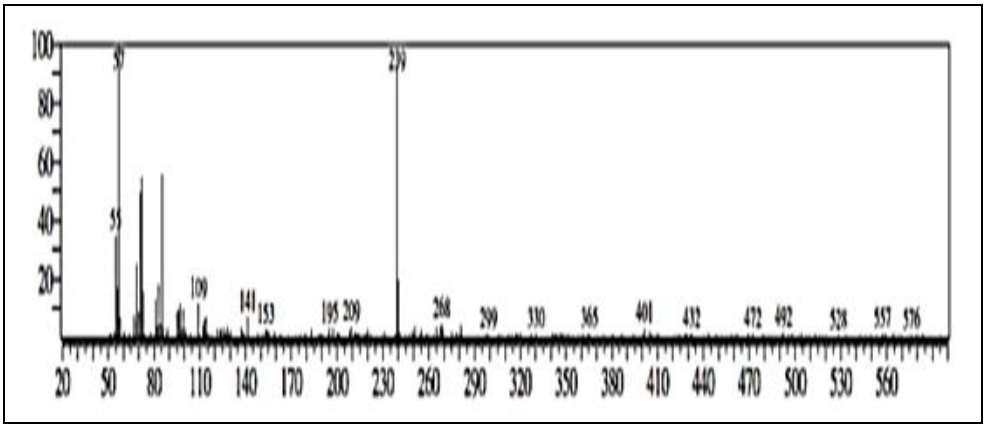
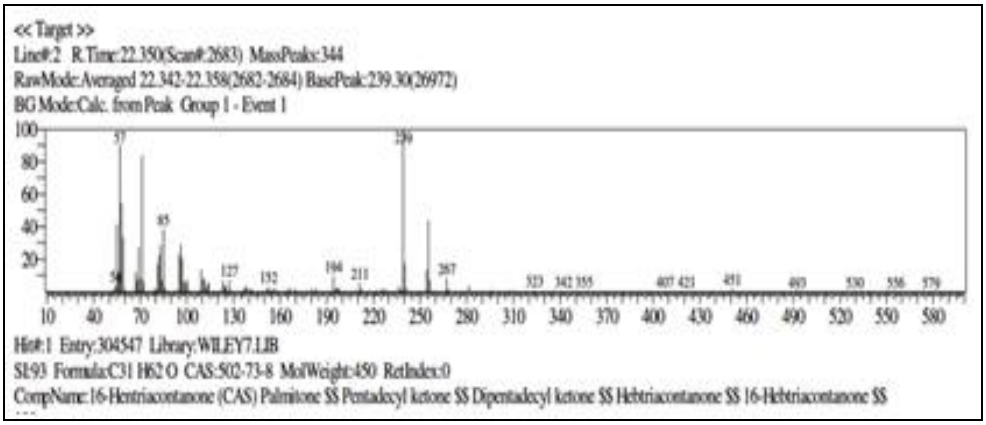


FIG. 1: GAS-CHROMATOGRAM OF *BREADFRUIT* LEAF EXTRACT



A. OCTADECAN-4-ONE



B. 16-HENTRICONTANONE

Effect of the Breadfruit Leaf Extract on Oxalate Levels: The research data showed that the levels of oxalate in the normal group (7.74 ± 0.177 mg/100 mg Kidney Weight). Induction of ethylene glycol 0.75% caused an increase in oxalate levels (14.44 ± 0.514 mg/100 mg KW) significantly, which meant that ethylene glycol is a precursor for oxalate formation **Table 2**.

TABLE 1: GC-MS ANALYSIS OF BREADFRUIT LEAF EXTRACT

Retention time	Compound	Molecular compound	Molecularweight
20.725	Octadecan-4-one, Ethylpentadecylketone, 3-Octadecanone	$C_{18}H_{36}0$	268
22.350	16-Hentriacontanone Palmitone, Pentadecyl ketone, Dipentadecyl ketone, 16-Hebtriacontanone	$C_{31}H_{62}O$	450
23.850	Octadecanoic acid, ethenyl ester, Vinyl stearate, Stearic acid, vinyl ester	$C_{18}H_{36}O$	310

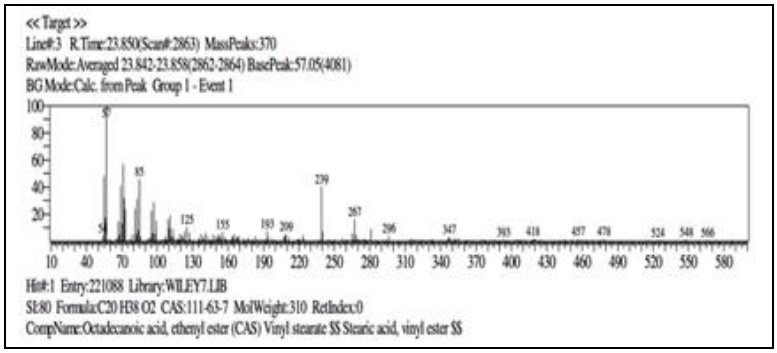
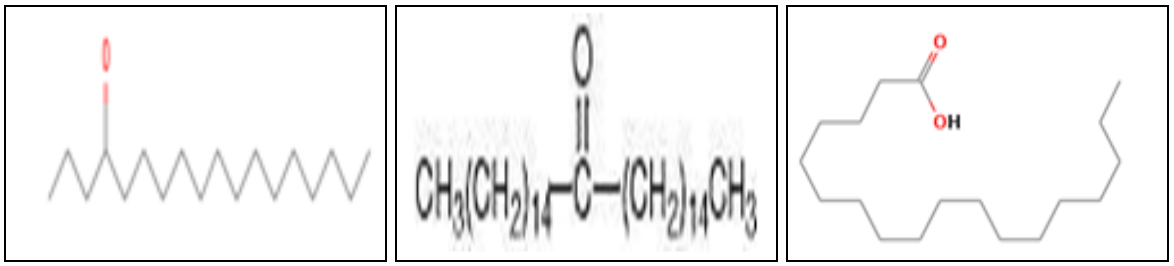


FIG. 2: MASS SPECTRUM CHROMATOGRAM OF (A) OCTADECAN-4-ONE, (B) 16-HENTRICONTANONE AND (C) OCTADECANOID ACID



OCTADECAN-4-ONE16-HENTRIACONTANONEOCTADECANOIC ACID

FIG. 3: THE STRUCTURE OF THE COMPOUNDS CONTAINED IN *BREADFRUIT* LEAF EXTRACT (PUBCHEM)

Effect of the breadfruit leaf extract on leukocyte differentiation: The data **Table 4** shows that scores of neutrophils, eosinophilia, lymphocytes, and monocytes in the normal group are higher than those of the reference. EG administration increased the expression of differentiation of neutrophils, eosinophilia, lymphocytes, and monocytes in all groups, while the lowest expression was shown in the BE3 group. This proves that the administration of breadfruit leaf extract 400 mg/kg BW reduces the expression of leukocyte differentiation.

DISCUSSION:

GC-MS Analysis of *Breadfruit* Leaf Extract Content: The chromatograms showed the greatest composition shown by peak 2 (16-Hentria-contanone palmitone) with percent area: 81.35%, followed by peak 3 (Octadecanoic acid): 9.80% and peak 1 (Octadecan-4-one): 8.85 %. The analysis of total flavonoid levels in breadfruit leaf ethanol extract was set at λ_{max} 510 nm, using the standard quercetin is obtained 7.60% mEQ.

TABLE 2: THE EFFECT OF BREAD FRUIT LEAF EXTRACT ON RENAL OXALATE CONCENTRATION

Groups	Mean ± SD (g/100g KW)
Normal	7,74 ± 0,177^{*2,3,4,5}
EG	14,44 ± 0,514^{*1,5}
BE1	14,20 ± 0,739^{*1,5}
BE2	13,54 ± 0,462^{*1,5}
BE3	11,55 ± 0,619^{*1,2,3,4}
pAnova	< 0,001

Note: EG: ethylene glycol; BE1: 100; BE2: 200, and BE3: 400 mg/kg BW; KW: Kidney weight * The mean difference is significant at the 0.05 level, p value< 0.05 Compared :1Normal; 2EG; 3BE1; 4BE2; 5BE3

TABLE 3: THE EFFECT OF BREAD FRUIT LEAF EXTRACT ON TOTAL LEUKOCYTE LEVELS

Groups	Leukocyte Total (x10^{3}cells/µL)	Normal (x10^3 cells/µL)**
Normal	16.76 ± 3.76^{*2}	7.30-12.66
EG	23.86 ± 7.94^{*1,3,4}	
BE1	18,64 ± 1,75^{*5}	
BE2	15.72 ± 3.76^{*2}	
BE3	12.22 ± 2.03^{*2,3}	
pAnova	0,00,008	

Note: EG: ethylene glycol; BE1: 100; BE2: 200, and BE3: 400 mg/kg BW * The mean difference is significant at the 0.05 level, p value< 0,05Compared 1Normal; 2EG; 3BE1; 4BE2; 5BE3 **Smith & Mangkoedidjojo, 198813

Effect of the *Breadfruit* Leaf Extract on Total Leukocyte Levels:

TABLE 4: LEUKOCYTE DIFFERENTIATION

Groups	Score ($x10^3$ cells/µL)				
	Neutrophil	Basophil	Eosinophil	Lymphocyte	Monocyte
Normal	3.08	0.0	0.54	10.52	0.39
EG	5.78	0.0	1.68	18.68	0.7
BE1	4.04	0.0	0.66	11.08	0.47
BE2	3.12	0.0	0.64	10.24	0.40
BE3	3.08	0.0	0.4	10.18	0.37
Reference*	0.22-1.57	0.0-0.5	0.01-0.16	1.41-7.11	0.03-0.18
Reference**	1.25-3.71	0.00-0.03	0.04-0.30	5.07-9.07	0.05-0.44

Note: * Giknis, and Clifford, 2006, Clinical Laboratory Parameters for Crl: WI(Ham)Rats14 ** Thrall *et al.* 2012, Veterinary Hematology and Clinical Chemistry [15]

Effect of the *Breadfruit* Leaf Extract on Oxalate Levels: Giving ethylene glycol will cause hyperoxaluria because ethylene glycol is metabolized by alcohol dehydrogenase to glycolaldehyde is then metabolized to glycolic, glyoxylic acid, and oxalic acid. Oxalate quickly settles with calcium to form insoluble calcium oxalate (CaOx) crystals.

$$2\ C_2O_4^{2-} + Ca^{2+}O\ Ca\ (C_2O_4)\ 2(Solid)$$

Triggers the process of hyperoxaluria deposition and CaOx crystals to make pathophysiological and morphological changes (tissue injury) in the kidney and affect the composition of urine [16].

Renal tubular lesions occur as a result of widespread CaOx deposition and the toxic effects of glycolic and glyoxylic acids [17]. Cellular tissue and its functions are disruption results in renal injury and inflammation, impairment and the inability of renal function and end-stage renal disease (ESRD) [18]. Urolithiasis or nephrolithiasis is a risk factor for kidney failure in primary hyperoxaluria [19].

One Way ANOVA Test Results in Fcalc: 70, 215, sig <0,001; CI: 95%, showed a significant difference between groups, The results of this study proved that the administration of ethanol extract of breadfruit leaves at a dose of 400 mg/kg bw significantly reduced and prevented the growth of CaOx stones ($p < 0.05$). Thus, this study proves breadfruit leaf extract at a dose of 400 mg/kg BW has an antinephrolithiasis activity.

Effect of the *Breadfruit* Leaf Extract on Total Leukocyte: Intra-tubular crystalline deposits can be detected quickly after day 1 in the medulla and cortex together by tubular injury, dilatation, interstitial inflammation, and regeneration. Inflammation is one form of response made by the body to protect against infection or injury. The initial symptoms of inflammation are changes in volume (and speed of blood flow resulting) and vascular permeability that occur in the area of inflammation. Leukocytes move from the bloodstream to the site of inflammation. When leukocytes migrate that occur through the vessel wall, they make changes in shape to pass through the endothelial junction, which is very narrow in size. This can provide information that leukocyte deformability is an important factor when the inflammatory process occurs [20]. This process is carried out by mast cells, eosinophils, basophils, and NK cells. Interstitial inflammation and tubular injuries usually occur and are observed in injury cases in the severe and chronic categories. Renal tubular epithelial cells can be an important support in the cause of kidney inflammation, secrete many inflammatory cytokines that are a response to immune and non-immune factors, and the presence of these cytokines 21 determines leukocyte infiltration.

Kidney inflammation is a cause of progressive kidney injury, which can cause glomerulonephritis, end-stage kidney disease, or acute or chronic kidney disease (CKD) [22]. As in the previous explanation, inflammation is a body's response that protects against infection or injury.

When the body's tissues treat the damage caused by infection or injury, the inflammatory response is triggered as an effort to begin the healing process. In some diseases, such as rheumatoid arthritis and atherosclerosis, the inflammatory response is

triggered incorrectly, making the inflammatory tissue damage healthy tissue [20].

Inflammatory infiltrate is composed of white blood cells, which leave the blood and then enter (infiltrate) cells inflamed. Inflammatory infiltrates cells are neutrophils, lymphocytes, and monocytes. Immigration of these cells into peripheral tissue is one of the main objectives of inflammation, bringing to the site of injury the immune system cells, which can fight infection and cleanse damaged tissue [20].

Leukocytes travel through the bloodstream to the site of inflammation, with the help of permeability of the vessel wall to change. Leukocytes have a duty to kill pathogens and make them disappear with phagocytosis. When leukocytes migrate and pass through the vessel wall, they make changes in shape to pass through the narrow endothelial junction. This can be interpreted as leukocyte deformability is important in the inflammatory process [20].

In this study, we found that the total leukocyte in each group were normal: (16.76 ± 3.76); EG: (23.86 ± 7.94); BE1: (33.34 ± 2.99); BE2: (15.72 ± 3.76) and BE3: (12.22 ± 2.03) × 10^3 cells / μL. According to the results of the one-way Anova test, Fcount: 4,650, p: 0,008, proving that there are significant differences between treatment groups, while the Post Hoc (Levene) test shows that the EG group (giving ethylene glycol 0.75%) increases leukocytes total, significantly different from the Normal group (p: 0.020); BE2 (p: 0.009), and BE3 (p: 0.001). The BE1 group was significantly different from the BE3 group (p: 0.033), and the BE3 group meaningfully different from the EG group (p: 0.001) and BE1 (p: 0.033). These data prove that breadfruit leaf extract at a dose of 400 mg/kg BW can reduce total leukocytes **Table 3.**

In line with previous studies, CaOx crystal deposition is a cause of inflammation and is responsible for attracting various inflammatory cells, including macrophages, leukocytes, and monocytes [23]. Inflammatory cells that enter the interstitium of the kidney cannot be known, but several chemotaxis factors and adhesion molecules are involved. Leukocytes (monocytes, neutrophils, and lymphocytes) infiltrate the kidney during inflammation, mediate kidney injury and subsequent sclerosis caused by pathology. Chemotactic is produced by kidney cells and can be easily found in the kidneys and urine during inflammation [24].

Our study found total flavonoid levels in an extract of 7.60% Meq; in a previous study, It is stated that flavonoids, quercetin-like compounds, exhibit anti-inflammatory effects by lowering indicators of chronic systemic inflammation (CSI). The anti-inflammatory effects of flavonoids are mediated to a large extent through blocking activities of the enzymes implicated in signaling pathways, especially protein kinase C and mitogen-activated protein kinases (MAPK), with downstream inhibition of transcription by inhibiting the transcription factor nuclear factor-kappa β (NF-κβ) and activator protein (AP)-1, decreasing levels of IL-1β and TNF-- in blood serum, and transcriptional activity of NF-κβ in blood mononuclear cells [25].

Peripheral Blood Mononuclear Cells (PBMCs) stimulated by lipopolysaccharide (LPS) treated with antioxidant display altered cytokine profiles, including decreased levels of pro-inflammatory mediators (Tumor Necrosis Factor (TNF)-α, interleukin and interferon (IFN)-γ. The anti-inflammatory properties of its antioxidants also extend to the production of some chemokines. This reduction in chemokines, in particular, will have an impact on the recruitment of monocytes and neutrophils to local inflammatory sites.

This antioxidant inhibiting effect is on the level of mRNA expression, which can occur due to a lack of natural killer activation (NF-κB) as occurs in the decrease in IκB-α mRNA that is present, IκB-α is a nuclear factor derived from kappa light which enhances enhancement polypeptide genes that occur in B-cell inhibitors, alpha. Antioxidants significantly inhibit the inflammatory response of PBMCs to LPS [26]. Thus, our research has shown the presence of flavonoids (antioxidants) in breadfruit leaf extract has an anti-inflammatory effect on urolithiasis-induced ethylene glycol rats (an oxidative stress-triggering compound).

Effect of AA Extract on Leukocyte Differentiation: Neutrophilsare leukocytes with the most numbers, around 60% of the total white blood cells. Neutrophilic leukocytes are white

blood cells that will enter the tissue that becomes inflamed when acute inflammation occurs. Neutrophils are anti-bacterial cells, which carry out the breakdown of bacterial cells through the release of lysosomal enzymes. Neutrophilia can occur physiologically or pathologically.

Physiological neutrophilia can arise when animals are stressed or overexcited [25]. Pathological neutrophilia often occurs in inflammatory conditions (inflammation), especially those that are acute. The highest average neutrophils were in the EG group (5.78×10^3 cells/µL) and the lowest is normal and BE3 group (3.08×10^3 cells/µL). Basohils have an essential role in hypersensitivity reactions, have functions similar to mast cells, and the ability to phagocytosis the causative agent of hypersensitivity [27]. In our study, all basophile values are zero because the basophil parameter is related to hypersensitivity, so there is no difference in values. In our research, there were no compounds suspected of causing hypersensitivity.

Eosinophilia not a disease, but it is a response to a person's immunity to infection. The higher levels of eosinophils (eosinophilia), the higher the immune response. As in this study, the highest standards of eosinophils are in the EG group (1.68×10^3 cells/µL) because inflammation in the EG group occurs due to the induction of ethylene glycol and without treatment by extract, and the lowest is BE3 group (0.4×10^3 cells/µL).

Lymphocytes accumulate somewhat later in the inflammatory process. B lymphocyte cells will differentiate into plasma cells that play a role in the humoral immune response to produce antibodies, whereas T lymphocytes will play a role in cellular immune response [28]. Their presence in very large numbers will indicate the continuation of the existence of antigens and can show the process of infection that occurs. Lymphocytes as antigen-producing types have various types (one type of specific antibodies per lymphocyte), which as a provider to recognize chemicals derived from foreign substances (distinguishing between self and non-self) and as one of the processes of regulating the immune response. An increase in the number of lymphocytes (lymphocytosis) can occur in problems with the immune system. The most common cause of decreased lymphocyte counts (lymphopenia) is corticosteroids. Conversely, if there is an increase in the number of lymphocytes, there is inflammation and stress there [14]. The highest lymphocyte values are in the EG group ($18,68 \times 10^3$ cells/µL) and the lowest, BE3 group (10.18×10^3 cells/µL) that means the administration of breadfruit leaf extract at a dose of 400 mg/kg body weight against the lowest lymphocyte score, the lowest risk of lymphocyte accumulation during the inflammatory process.

Monocytes are a phagocytic cell that circulates in the blood. An equivalent type of cell, called a macrophage, is connective tissue precipitation. Monocytes/macrophages carry out digestion and eat dead cells, foreign microorganisms, and other tissue debris. They perform closely with lymphocytes as part of recognizing and destroying foreign substances. An increase in the number of monocytes (monocytosis) can occur as a stress response in ruminants; however, monocytosis can also occur in inflammatory conditions.

Decreasing the number of monocytes (monocytopenia) can be caused by endotoxemia, chronic, and acute inflammation caused by various causative agents [14].

The data shows that the highest monocyte value lies in the EG group (0.7×10^3 cells/µL) because ethylene glycol causes oxidative stress, which triggers inflammation. The lowest is the BE3 group (0.37×10^3 cells/µL), and in this group proves that the breadfruit leaf extract dose of 400 mg/kg BW affects the monocyte score.

The data shows that scores of neutrophils, eosinophilia, lymphocytes, and monocytes in the normal group are higher than those of the reference. EG administration increased the expression of differentiation of neutrophils, eosinophilia, lymphocytes, and monocytes in all groups, while the lowest expression was shown in the BE3 group. This proves that treatment with *breadfruit* leaf extract of 400 mg/kg BW influences leukocyte differentiation.

CONCLUSION: Our research shows that according to GC-MS analysis, *breadfruit* leaf extract contains Octadecan-4- one, 16-Hentria-contanone, and Octadecanoic acid compounds, preventing calcium formation oxalate kidney stones and reduce the total leukocyte and inflammatory

markers. The optimal dose of breadfruit leaf extract is 400 mg/kgbw.

ACKNOWLEDGEMENT: This research was supported by Prof. Suparwi Animal Hospital and the Animal Research Ethics Committee at the Faculty of Veterinary Medicine, Gajah Mada University, Yogyakarta.

CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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How to cite this article:
Susilo J, Purwanto B, Doewes M, Indarto D and Muktiwi DR: *Breadfruit* leaf extract composition and its effect on oxalate and leukocyte profiles in hyperoxaluric rats induced by ethylene glycol. Int J Pharm Sci & Res 2021; 12(8): 4525-34. doi: 10.13040/IJPSR.0975-8232.12(8).4525-34.