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Original Research Paper

Effect of Guava Fruit Aqueous Extract on Haematological Profile of Carbon Tetrachloride Induced Rat

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Abstract: *Psidium guajava* or guava have many potential value, including as antioxidant. *Psidium guajava* fruits have been reported that contain essential oil, flavonoid, polyphenol, ascorbic acid, dan tocopherol. This study aims to investigate weight gain and heamatological alteration as an effect from administration of red guava fruit extract in male wistar rats after carbon tetrachloride. This study was experimental design using male wistar rats as an experimental animals. The results of the study were red guava fruit aqueous extract can control weight gain in model animal, increasing of LYM, MCMC dan WBC. It indicated that *Psidium guajava* fruit is potentially be able to be used as an pharmaceutical product because of its antioxidant ability.

Keywords: Antioxidant, CCl_4, guava, haematology.

Introduction

Psidium guajava is known as super fruit because of its nutritional value. *Psidium guajava*, or commonly mentioned as guava is one of agriculture commodity that easily found in Indonesia. Based on Badan Pusat Statistik, in year 2023, Indonesia had produced guava fruit of 404.654 tons, which means that it abundantly available in this region (BPS, 2023). In many country of Asian states, including Indonesia, guava usually consume as fresh fruit or juice because of its good flavour, aroma, and reddish color.

Guava, especially red guava, is reported containing of ascorbic acid and several phytochemical constituens that play a beneficial roles in human health (Tousif et al., 2022). It is also known of its high fiber, protein, fat, and mineral such as potassium, zinc, iron, calcium, sodium, and selenium(Asif et al., 2022; Yousaf et al., 2021). Traditionally, guava is widely used as anti-diarrhea (Asif et al., 2022; Gutierrez-Montiel et al., 2023), anti-trombositopenia (Hosea et al., 2018; Mahalaksmi et al., 2024), hypo-glicemic, hypo-lipodemic, antibacterial (Asif et al., 2022), and antioxidant(Gupta et al., 2011;Anand et al., 2016; Asif et al., 2022;).

Many studies about guava had been carried out on leaves and fruits. As well as the leaves, guava fruits have been reported containing various phytochemicals such as essential oil (Harahap, 2021; Shukla et al., 2021), myrcetin, quercetin, apigenin, gallic acid(Shukla et al., 2021), ascorbic acid, and tocopherol(Anand et al., 2016; Harahap & Situmorang, 2021; Masud Parvez et al., 2018). Based on the evidences, guava is known containing a glycoside pholyphenol(Asif et al., 2022). Its constituents are investigated as antioxidant properties because of its ability to scavenge reactive oxygen species and enhance tissue restoration from oxidative damage (Meles et al., 2021).

Oxidative stress happen when there is imbalance between reactive oxygen species (ROS) and intracellular antioxidant enzymes. It leads to oxidative damage of the cell because ROS can oxidize all of parts of the cell and cause the cell to function improperly(de Almeida et al., 2022). If the body can not boost the production

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of antioxidant, antioxidants agent can be used to modulate intracellular redox stability(Chaudhary et al., 2023). Guava fruit can be one of the antioxidant agents that help body stabilizing redox equilibrium.

Based on the previous studies, guava fruit has been extracted with various solvent. But, extracting with water is considered to be more convenient because its polyphenol constituent is in the glycoside form. Furthermore, water is a non toxic, abundant, and '*cheaper'* solvent compare to the other organic solvent(Gallina et al., 2022). Preliminary study about guava aqueous extract had been carried out to determine total flavonoid content (TFC) (Rahmawati et al., 2023) and total phenolic content (TPC) (Rahmawati et al., 2024). Previous studies reported that red guava fruit aqueous extract (RGFA) contained TFC of 3,292±0,155mgQE/g and TPC of 4,037±0,019 mgGAE/g (Rahmawati et al., 2023, 2024). Many published studies tested guava fruit extract on radical scavenging activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) and concluded that guava fruit potentially can act as an antioxidant (Ademiluyi et al., 2016; Fitriana & Royani, 2020; Lahlou et al., 2022; Rusmiyati et al., 2023).It is reported to be able to elevate hemoglobin(Utami & Farida, 2022), thrombocyte (Hosea et al., 2018), and suppressed blood glucose level (Maa'idah & Pamungkas, 2023). It also states that dietary antioxidants can elevate white blood cells (Wambi et al., 2008).

This study aims to investigate the effect of RGFA in male wistar rats after carbon tetrachloride (CCl_4) induction. Carbon tetrachloride is colorless, sweet, fireproof and volatile liquid substance that very toxic to kidney, liver, brain, lung and other organ because of its ability to cause lipid peroxidation (Unsal et al., 2021). Induction of CCl_4 to experimental animal leads to trigger an oxidative damage that can be observed by examinating haematological parameter. As we know, blood reflect a pathological condition after exposure to some toxicants (Etim, 2014). Haematological components like white bloods, red bloods, and platelets often disrupted.

Oxidative stress is reported can decrease white blood cells, especially lymphocyte viability because of DNA damage, lipid peroxidation, and disruption of mitochondrial function(Gaharwar et al., 2017). It also can reduce hemoglobin and change red blood cell structure because red blood cell carries many oxygen molecule. In order to stabilize haematological parameters, guava as an antioxidant agents is given. It expected to be able to protect body from toxicant-iduced oxidative stress.

Materials and Methods

Materials

Red guava fruit, Aquadest, Maceration vessel, Oven, Glass Rod, Male Wistar Rat, Pellet, Water, Virgin Coconut Oil, Carbontetracloride, Vitamin C, Mikro-hematocrit cappilary tubes, Oral Probe, Diatron Abascus 3CT Analyzer, Hematology reagent, EDTA Tube.

Research Design

This study was experimental design research that conducted in Pharmacology Laboratory of Sekolah Tinggi Ilmu Kesehatan Nasional in September and October 2024.

Preparation of Red Guava Fruit Aqueous Extract

Dried red guava fruits was macerated with aquadest with ratio 1:10 (w:v) for 5 days in a dark condition. Stirring was done once a day to avoid saturation (Kementerian Kesehatan Republik Indonesia, 2017). The mixture then filtered with Whatman no 4 filter paper. The pooled filtrate was evaporated using oven in 40°C until a thick extract was form, which is hereafter referred to as Red Guava Fruit Aqueous Extract (RGFA).

Experimental Animals

Adult male Wistar rats with 180-200 gram weight and between 8-10 weeks of age were used. Rats were purchased from CV Rat House Bussiness husbandry then had been adapted for at least 3 weeks before treatment in a controled condition. Pellets was used as a daily feeding and access to the water *ad libitum* was given. The use of experimental rats were ethically approved under number 149/EC/KEPK/XI/2024.

Experimental Design

Experimental design was conducted according to the Guidelines for Preclinical

Pharmacodynamic Testing of Traditional Medicines (Badan Pengawas Obat Dan Makanan, 2021). Male wistar rats were grouped randomly into 6 groups. Each group consisted of 5 rats and treated as follow,

- Group 1: Normal Control, received no extract and no CCl_4
- Group 2: Negative Control, received no extract but induced with CCl_4 (0,05 cc/kgBB diluted in VCO)
- Group 3: Positive Control, received vitamin C (80 mg/kgBW diluted in water) and induced with CCl_4
- Group 4: 50 mg/kgBW RGFA administration and induced with CCl_4
- Group 5: 150 mg/kgBW RGFA administration and induced with CCl_4
- Group 6: 250 mg/kgBW RGFA administration and induced with CCl_4

Oral administration of vitamin C and RGFA were done using oral probe for 14 days. Stress oxidative induction by CCl_4 started on day 10 until 14, ten (10) minutes after vit C and RGFA (depend on treatment group). Rats then were starved overnight for blood collection. Body weight was measured in first day and last day of the treatment.

Blood Collection

Blood was collected from retro-orbital vein using micro-hematocrit capillary tube and transfered into EDTA tube for subsequent haematological investigation.

Haematological Investigation

Haematological investigation was done automatically using Diatron Abascus 3CT Analyzer. The result then documented for the next analysis.

Data Analysis

Data was presented as mean±SD and statistically analyzed with Kruskall Wallis Test to determine the significant difference between the treatments. Statistical analysis was done using IBM SPSS 22.

Results and Discussion

Experimental Animal Weight Gain

RGFA administration after CCl_4 induction affected on experimental animals weight gain as seen at Table 1. Statistically, we found that the treatments were not statistically different (p value 0,357 > 0,05), but it did not mean the treatments resulted 'zero different'. It is because descriptively, there were a different mean between treatment groups as seen as Table 1.

Table 1. Average body weight (g) before and after treatment with CCl_4, Vitamin C, and RGFA in various doses (n=5)

Treatment	Baseline (g)	After Treatment (g)	Weight Gain (%)
		mean±SD	
N	178,6±22,33	214,4±15,75	20,04
NC	182,4±12,45	219,4± 9,99	20,29
PC	167,8±23,80	199,8±16,55	19,07
RGFA 50	159,8±10,11	195,2±13,56	22,15
RGFA 150	164,4± 6,34	186,0± 8,32	13,14
RGFA 250	165,8± 7,25	196,8± 8,25	18,70

N: Normal; NC: Negative Control (CCl4); Positive Control (Vitamin C); RGFA 50,150,250: Red guava fruit aqueous extract in dose 50 mg/kgBW, 150 mg/kgBW, 250 mg/kgBW

Table 1 shows that all of the treatment groups gained weight for 14 days. The biggest weight gain is occurred in treatment group of RGFA with dose 50 mg/kgBW followed by negative control and normal control. Despite the high weight gain in treatment group of RGFA 50, the trendline was decreased (Figure 1). It showed that animal weight gain in RGFA 150, positive control, and RGFA 250 was more controlled in 13%, 18,7%, and 19%, respectively.

It was consistent with the study about relationship between antioxidant and weight gains, which mentioned that antioxidant agent with right nutritional composition helps body reduce fat and avoid excess weight gain (Martinez-Saez et al., 2014). It also consistent with previous study about guava by-product consumption that is effective to control adipose homeostatis because of its antioxidant properties(Batista et al., 2021).

Figure 1 shows that percentage of rat body weight increased in carbon tetrachloride induced rat compared to normal control. It showed that carbon tetrachloride affected the oxidative stress status of experimental animal. It induced acute oxidative damage and lipid peroxidation that lead

to oxidative injury (Unsal et al., 2021). Oxidative stress increasing adipocytes formation by elevating preadipocytes proliferation, differention, and development of mature adipocytes (Manna & Jain, 2015). That can lead to alteration of food intake which cause obesity.

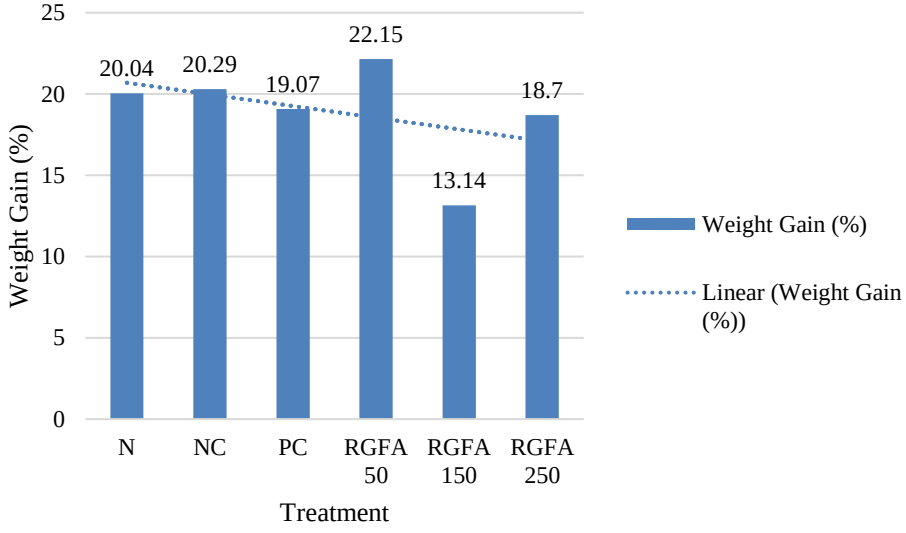

Figure 1. Histogram shows degression of weight gain after treatment with CCl_4, Vitamin C, and and RGFA in various doses (n=5)

Table 2. White blood cell, Hemoglobin and Hematocrit level after treatment with CCl_4, Vitamin C, and RGFA in various doses (n=5)

Treatment	LYM (10^3/µL)	MD (10^3/µL)	GRA (10^3/µL)	WBC (10^3/µL)	MCH (pg)	MCHC (g/dL)	HGB (g/dL)	MCV (fL)	HCT (%)	RBC (10^6/µL)
	mean±SD									
N	7,9±2,40	1,2±0,39	2,6±0,45	11,6±2,64	19,5±0,34	36,8±0,48	36,8±0,41	52,9±1,21	46,8±1,25	8,8±0,33
NC	6,9±1,14	1,1±0,13	2,8±0,65	10,8±0,56	19,7±0,40	35,8±4,71	17,1±0,75	52,4±1,40	45,1±1,89	8,6±0,40
PC	6,7±2,22	1,2±0,34	3,5±1,90	11,4±3,00	19,9±1,77	36,9±1,51	15,7±1,29	55,0±2,95	42,7±3,63	6,3±3,17
RGFA 50	6,6±1,37	1,3±0,54	2,1±0,77	9,5±1,47	19,5±1,37	36,3±2,43	16,7±1,21	53,9±4,94	46,0±2,57	8,6±1,09
RGFA 150	9,7±2,38	1,2±0,26	2,3±1,09	13,1±2,92	18,4±0,79	36,9±1,35	15,9±1,29	49,9±1,34	43,4±4,14	7,1±3,45
RGFA 250	12,8±6,35	1,6±0,55	2,9±0,95	17,3±6,89	19,6±1,86	37,0±1,69	15,6±1,99	52,8±2,73	42,3±6,82	8,1±1,66

N: Normal; NC: Negative Control (CCl4); Positive Control (Vitamin C); RGFA 50,150,250: Red guava fruit aqueous extract in dose 50 mg/kgBW, 150 mg/kgBW, 250 mg/kgBW; LYM: Lymphocytes; MD:Mid-cell leukocyte; GRA: Granulocytes; WBC: White blood cells; MCH: Mean corpuscular hemoglobin; MCHC: Mean cospuscular hemoglobin concentration; HGB: Hemoglobin; MCV: Mean corpuscular volume; HCT: Hematocrit; RBC: Red blood cells.

Experimental Rats Haematology

Table 2 shows of some haemetological components, especially white blood cells, haemoglobin, and red blood cells. Increasing of total white blood cells in Table 2 was accompanied with increasing of Lymphocyte (LYM) and Mean Corpuscular Hemoglobin Concentration (MCHC). Statistically, all of the parameter was >0,05, which means it was not statistically different. However, as seen in Figure 2, it showed that there was an increasing of lymphocyte based on dose-dependent manner. Meanwhile, based on Figure 2, mid-cell leukocyte and granulocyte showed that there were not difference between treatments. Lymphocyte in negative control decreased when compared to normal control. It was very reasonable because CCl_4 which can trigger reactive oxygen species production, can decrease lymphocyte viability because of oxidative stress (Kulaksizoglu & Kulaksizoglu, 2016).

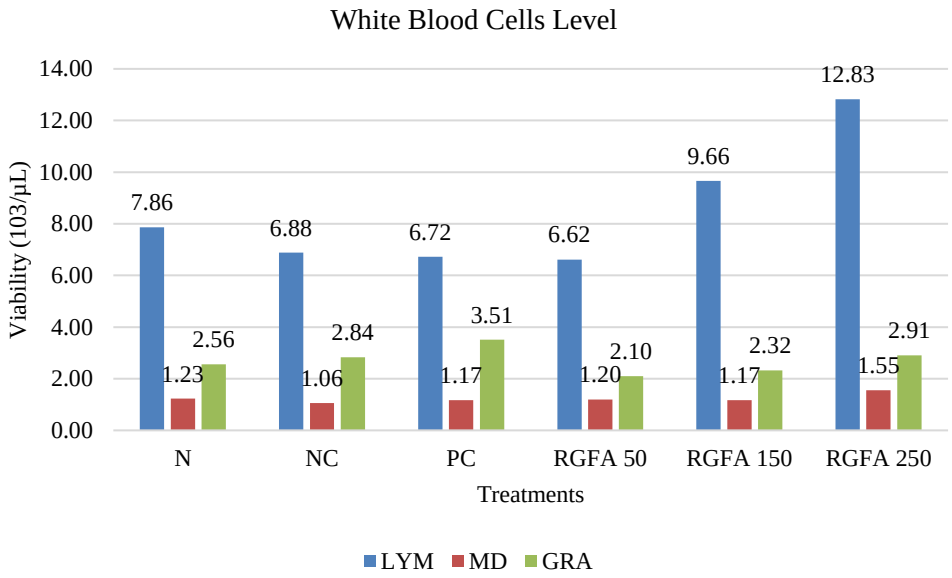

Figure 2. Histogram shows the effect of RGFA administration on white blood cells level of CCL_4 induced rats (n=5). N: Normal; NC: Negative Control (CCl4); Positive Control (Vitamin C); RGFA 50,150,250: Red guava fruit aqueous extract in dose 50 mg/kgBW, 150 mg/kgBW, 250 mg/kgBW; LYM: Lymphocytes; MD:Mid-cell leukocyte; GRA: Granulocytes

Lymphocyte start to increase when it treated with RGFA in 150 mg/kgBW and 250 mg/kgBW, indicating that there was a radical scavenging activity that protect lymphocyte from lipid peroxidation. Vitamin C and red guava fruits extract reduce reactive oxygen species by stabilizing them, so they can not easily oxidize other compounds (Bolin et al., 2012). It showed that guava potentially become immunostimulatory agent for the future (Laily et al., 2015). Guava fruits contain essential oil, myrcetin, quercetin, apigenin, gallic acid, ascorbic acid, and tocopherol can act as an antioxidant agents. Those antioxidant agents will reduce superoxide anion and eventually maintain or even elevate lymphocyte viability (Sudewa & Arijana, 2018).

The other result related to heamatological parameter was MCHC value. Table 2 showed that MCHC in negative control is 35,8±4,71 g/dL which was lower than normal control. Since MCHC is an indicator for average amount of hemoglobin in a single red blood cell, lower MCHC in Table 2 showed that there was a decreasing of iron in hemoglobin. Acute oxidative stress by CCl_4 induction can disrupt iron homaeostatis (Galaris et al., 2019). We found that administration of RGFA in dose dependent manner could elevate MCHC in 0,52 – 1,18 g/dL, respectively. It was relevant to previous studies that guava fruit can increase hemoglobin in red blood cell (Hardimarta et al., 2018; Utami & Farida, 2022). Guava fruits that also containing mineral, including iron (Yousaf et al., 2021; Asif et al., 2022), together with flavonoid, phenolic and ascorbic acid could helps body maintain iron bioaviability and prevent anemia (Utami & Farida, 2022; Rani et al., 2024). Further study about relationship between guava fruits in iron homeostatic need to be conducted for farther and evident evidence.

Conclusion

Red guava fruit aqueous extract administration after CCl_4 induction affect animal weight gain and haematological parameters especially in lymphocyte, white blood cell and average hemoglobin concentration level in experimental animals by increasing them in dose dependent manner. In the other hand, mid-cell leukocyte and granulocyte are not showing difference between treatments.

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