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CEK_Nurani,Darmawan,Akrom, Guntarti,Warsi,Edityaningrum_ eukocytes, lymphocytes, BUN, SGOT, SGPT, Hibiscus sabdariffa

by Nurani,darmawan,akrom,guntarti,warsi,edityaningrum Effect Of
Ethanolic Extract Of Rosella

Submission date: 25-Oct-2022 01:36PM (UTC+0700)

Submission ID: 1934802935

File name: Herbabolonia_68_3_2022.pdf (1,015.72K)

Word count: 5028

Character count: 25839

Received: 2022-02-04

Accepted: 2022-04-20

Available online: 2022-09-30

DOI: 10.2478/hepo-2022-0018

EXPERIMENTAL PAPER

Effect of ethanolic extract of rosella (*Hibiscus sabdariffa* L.) on vital signs, kidney, and liver safety (*ahead of print*)

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Summary

Introduction: Rosella (*Hibiscus sabdariffa* L.) extract is often used as immune-stimulant because it contains flavonoids, especially anthocyanin and quercetin with antioxidant activities.

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Herba Pol 2022; 68(3): 1-9

Objective: This study aimed to determine the safety of the rosella extract consumed in the form of capsules on the vital signs, haematologic parameters as well as kidney and liver function.

Methods: This research was conducted using clinical trial pre- and post-test design in healthy participants. There were 21 healthy participants (52% male, age ranged 8–45) consuming rosella capsules for thirty days, in a dose of 500 mg extract daily. Leukocytes, lymphocytes, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and vital signs were consecutively evaluated on days 0, 31, and 45, respectively. The Wilcoxon and paired sample t-test were used to compare the parameters among the evaluated times.

Results: The result showed that no significant difference for all parameters among the three time points ($p > 0.05$).

Conclusion: These findings suggested that the administration of ethanolic extract of rosella is potential safe and does not negatively affect the vital signs, haemoglobin, leukocytes, lymphocytes, BUN, SGOT, and SGPT.

Key words: *leukocytes, lymphocytes, BUN, SGOT, SGPT, Hibiscus sabdariffa*

Slowa kluczowe: *leukocyty, limfocyty, BUN, SGOT, SGPT, Hibiscus sabdariffa*

INTRODUCTION

Rosella (*Hibiscus sabdariffa* L) contains antioxidant compounds capable of enhancing the immune system by maintaining cell damage due to its ability to absorb excessive ultraviolet light, therefore, it protects body cells from free radical changes [1]. The antioxidant compounds include metabolite of phenolics and flavonoids [2] such as hibiscitrin (hibiscetin-3-glucoside), quercetin, gossytrin, sabdaritrin, gossypitrin, gossypetin glucosides, and luteolin [3], fatty acid [4], alkaloids, steroids, triterpenoids, saponins, tannins, anthocyanins, and β -carotene [5, 6]. The antioxidant activity of rosella are mainly due to red pigment of anthocyanin, increasing the scavenging activity of free radicals [7]. Anthocyanins also stimulated the production of erythropoietin, which promotes the formation of red blood cells [8]. Thus, it affects the number of leukocytes and lymphocytes in the immune system [9].

Antioxidants maintain the immune system in human body. Every deficiency in the antioxidant defence system make the human body more susceptible to oxidative stress [10] due to imbalance between reactive oxygen species (ROS) and endogenous antioxidant. Induction of ROS can damage lymphocytes in healthy humans. In the immune system, leukocytes play important role in producing, transporting, and distributing antibodies, as a part of the immune response to antigens [11]. Immune reactions to microorganism attacks, foreign macromolecules, and cancer cells are initiated by lymphocytes that are the granular form of leucocytes. Lymphocytes specifically recognize and respond to

foreign antigens and also serve as a mediator of humoral and cellular immunity [12].

The preclinical study revealed that rosella provided therapeutic potential of immune-stimulator at an optimal dose of 50 mg/kg BW [12]. Therefore, its use in humans is feasible. Clinical parameters that can be used in describing the tolerated dosage is haematologic, kidney, and liver changes which is a phase 1 clinical test parameter [13].

The ethanol extract of rosella petals has a lethal dose 50% (LD_{50}) toxicity value of 850.90 mg/kgWB in Sprague Dawley mice in the category of ring toxicity and has no effect on the enzyme activity of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) [14]. These enzymes are specific indicators for determining liver cell damage. Kidney function can also be a parameter in determining the safety of medicinal plants [15]. The examination of blood urea nitrogen (BUN) is one of the markers of renal function, which can be used to evaluate kidney function, although, the elevated BUN levels do not actually reflect renal function [16]. Haemoglobin (Hb) serves as the oxygen-carrying substance present in the erythrocytes [17]. Increased or high Hb levels are generally associated with increased amounts of erythrocytes produced in bone marrow with raw materials of iron (Fe), amino acids, vitamin B₁₂, folic acid, B₂, B₆, vitamin C, vitamin E, minerals (cobalt and copper), and the hormone erythropoietin. A decrease in Hb level may indicate a haematological disorder of anaemia [18].

This research aimed to assess the safety of the rosella extract consumed in the form of capsules on

the vital signs, hematologic parameters as well as kidney and liver function.

MATERIAL AND METHODS

Materials

The sample used in this study was obtained from Natura (Food and Nutratecal Company; Jakarta, Indonesia) with brand name of rosella PE[®] with product code: 5055C and batch number: RH 162703.

Place and time of research

This research was conducted at a private clinic in Yogyakarta, Indonesia from April 3 to May 17, 2017, for 45 days. The ethical clearance, or ethical approval, regarding the clinical study was obtained from Muhammadiyah University of Yogyakarta (approval number: 255/EP-FKIK-UMY/IV/2017).

Research instrument

Instrument used for measuring lymphocytes is flow cytometry, Hb and leukocyte count were haematological analyser, autoanalyser photometer SGOT-SGPT examination, and BUN examination using spectrophotometer with Modiff-Berthelot method.

Health checkup and preparation

Medical examination

This research was conducted by using pre- and post-treatments research design involving 21 volunteers consisting of 11 males and 10 females selected based on inclusion and exclusion criteria [19, 20]. The inclusion criteria included doctor's certificate confirming a health status, age 18–45, BMI 18–30, non-smokers, and signed consent. Health criteria included vital signs, blood pressure, body temperature, heart rate (HR) and respiration rate (RR). The medical examination results were stated in a health certificate supported by clinical laboratory examination including routine haematology, liver function, kidney function, history of disease, and physical examination. Exclusion criteria included pregnant women, consuming other drug during research duration, and using other herbs during the study.

Administration of rosella extract capsule

Rosella extract capsule was given at a dose of 500 mg/day (Product code: 5055C, Batch: RH162703). The capsules were administered to volunteers for 30 days. Haemoglobin, leukocytes, lymphocytes, BUN, SGOT, SGPT and vital sign were examined on day 0 (before treatment), 30th day (after 30 days of consuming rosella capsule) and 45th day (15 days after not consuming rosella capsule) [19].

Preparation of blood sampling

Blood samples were taken from the median cubital vein or brachial vein using 5 cc syringe for 4 cc blood. Then, the sample was stored in a centrifugal micro tube containing EDTA as a coagulant. The separation of plasma was carried out by centrifugation at a rate of 3,500 rpm for 2 hours so that the transparent yellow liquid (serum) occurred. If the serum was not used immediately, then the liquid was stored in a freezer in a temperature of –200°C [21, 22].

Examination of haemoglobin, leukocytes, lymphocytes, BUN, SGOT and SGPT

Haemoglobin and leukocytes

Blood samples on the EDTA tube were analysed using an automated haematology analyser [23].

Lymphocytes

The results of blood samples on the EDTA tube were stored in the falcon tube as much as 50 µl. The tube was added with each 5 µl tritest CD3APC/CD-4PerCP/CD25PE reagent. It was then homogenized using vortex for 1 minute. It was then incubated for 15 minutes with temperature 20–25°C in a dark room. Then, 450 µl of solution of lytic agent was added, followed by an incubation for 15 minutes in a temperature of 20–25°C in the dark room. After the incubation period was completed, an analysis was done using FACS flow cytometer [22].

Blood urea nitrogen (BUN)

The levels of BUN were determined by the Modiff-Berthelot method. The volunteer's blood sampling was fed into a tube using a microphone. A-0.5 ml colour reagent was added to the bottle labelled: unknown,

control, standard, blank. A-10 µl was added to the sample into appropriate bottle. A-0.5 ml of enzyme reagent was added to all bottles. Then, it was mixed well and incubated at 37°C for 5 minutes. A-2.0 ml base reagent was added. The mixture was then stirred and incubated at 37°C for 5 min. The wavelength was set at 630 nm and the zero of the spectrophotometer with blank. The concentration of unknown samples was read directly using standard (25 mg/dl) [24].

Serum glutamic oxaloacetic transaminase (SGOT) – serum glutamic pyruvate transaminase (SGPT)

A total of 1000 µl of reagents were introduced into the test tube. The reagents used were ready-to-use reagents consisting of SGOT and SGPT reagents. Furthermore, 100 µl serum was added and shaken for 1 min in a water heater at 37°C. Spectrophotometer readings were performed at a 340 nm wavelength with a conversion factor of 1745 to obtain SGOT and SGPT levels. SGOT and SGPT levels were expressed in units/litre (U/L) [25].

4 Data analysis

The data were presented as mean ±SD. The normality test was carried out using Shapiro-Wilk. If data

are normally distributed, the means of pre- and post-treatments were subjected to paired t-test, while for the non-normally distributed data, two medians were analysed using a Wilcoxon test at a 95% confidence level. All statistical test were carried out using IBM SPSS 23 statistics.

RESULTS AND DISCUSSION

In this study, Hb, leucocytes, lymphocytes, BUN, SGOT, and SGPT levels in male and female volunteers were found to be different in mean levels at day 0, 30 and 45. Clinically, however, the levels were still within the normal limits (tab. 1, tab. 2).

Effect of ethanolic extract capsule of rosella on vital signs

Table 3 showed the results of healthy volunteers' check-ups after 30 days of administration of rosella capsules and after not consuming rosella capsules for 15 days. It showed that there was no significant difference in vital signs. Vital signs of the 31st day were within the normal range. In this study, there was no significant difference after the administration of rosella capsule and after not being given it on systolic blood pressure value, diastolic blood

Table 1. Results of haemoglobin, leukocytes, lymphocytes, BUN, SGOT, and SGPT on healthy volunteers (n=21)

Parameters	Mean ±SD			Normal value [26]
	Day 0	Day 31	Day 45	
Male				
Haemoglobin [g/dl]	14.29±1.59	14.49±1.64	14.77±1.57	13–18
Leukocytes [10 ³ u/l]	7.31±1.96	6.52±1.34	7.10±0.87	4–11
Lymphocytes [%]	37.92±10.2	32.84±6.71	32.38±7.47	15–45
BUN [mg/dL]	21.81±5.79	19.90±5.41	23.90±5.37	10–50
SGOT [U/L]	23.69±4.45	21.18±4.02	20.45±3.64	10–36
SGPT [U/L]	13.81±5.87	12.72±4.22	12.54±3.93	9–43
Female				
Haemoglobin [g/dl]	12.01±1.06	12.39±1.54	12.03±1.70	12–16
Leukocytes [10 ³ u/l]	6.70±1.24	6.29±1.88	6.86±2.19	4–11
Lymphocytes [%]	32.25±9.61	31.32±1.17	28.48±5.59	15–45
BUN [mg/dl]	21.44±5.81	22.89±8.31	21.00±6.82	10–50
SGOT [U/L]	19.80±5.39	19.80±5.39	19.80±5.39	10–36
SGPT [U/L]	10.10±4.70	11.20±4.51	11.00±3.49	9–43

Note: BUN: blood ureum nitrogen; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvate transaminase

Table 2.

Results of paired t-tests for changes of haemoglobin, leucocyte, lymphocyte, BUN, SGOT and SGPT on healthy volunteers (n=21)

Examination	p-value (male)			p-value (female)		
	d1	d2	d3	d1	d2	d3
Haemoglobin	0.266	0.113	0.066	0.460	0.570	0.414
Leucocyte	0.130	0.090	0.624	0.420	0.230	0.755
Lymphocyte	0.131	0.776	0.047*	0.818	0.332	0.049*
BUN	0.448	0.061	0.285	0.321	0.428	0.486
SGOT	0.161	0.531	0.070	1.000	0.848	0.725
SGPT	0.545	0.913	0.165	0.733	0.933	0.634

Note: Paired t-test: d1 = day 0 and 31; d2 = day 31 and 45; d3 = day 0 and 45

Table 3.

Results of vital check-ups on healthy volunteers (n=21)

Examination	Normal value	Value (n=21)		p-value
		Day 30	Day 45	
Systolic [mmHg]	≤20	115.95±8.45	115.00±6.32	0.43
Diastolic [mmHg]	≤80	75.90±6.44	74.57±7.80	0.43
Pulse [x/ment]	70–80	78.86±7.05	77.48±4.16	0.27
Respiratory rate (RR) [x/ment]	16–20	17.71±1.82	18.38±1.02	0.15
Temperature [°C]	36.1–37.2	36.16±0.35	36.65±0.40	0.01

Note: Wilcoxon test ($p < 0.05$); Day 30: after administration of ethanol extract capsule of rosella petals; Day 45: after 15 days do not consume ethanol extract capsule of rosella petals

pressure, pulse, respiration ($p > 0.05$). Previous research showed that rosella plays a role in maintaining blood pressure in healthy volunteers by decreasing the activity of renin-angiotensin (RAS) and the active vasodilator metabolite of bradykinin (des-Arg(9)-bradykinin). Rosella tea effectively lower blood pressure in patients with mild hypertension. There was no significant difference in the body temperature ($p < 0.05$). Literature reviews suggest that flavonoid contained in rosella has the ability to inhibit cyclooxygenase reactions which can affect the biosynthesis of prostaglandins, a mediator of febrile formation.

Effect of ethanolic extract capsule of rosella on haemoglobin, leucocytes and lymphocytes

The comparison of haemoglobin, leukocyte, and lymphocyte levels in male and female volunteers on day 0 and day 31 in table 2 showed that there is no significant difference ($p > 0.05$). This indicates that ethanolic extract of rosella at given doses does not

affect the haematology of healthy human blood during and after administration. Table 1 revealed the increased levels of haemoglobin but within normal limit. The anthocyanin present in rosella can stimulate the production of erythropoietin thus affecting the formation of red blood cells. The erythrocytes are then synthesized to produce haemoglobin [27]. The result showed that iron mineral levels as well as vitamins from rosella also help increase the number of Hb volunteers. Vitamin C is needed to increase iron absorption. While Fe binds to haemoglobin to carry oxygen throughout the body. High vitamin C and Fe content, in rosella can increase the number of erythrocytes and Hb levels in white blood of anaemic mice. Vitamin C and Fe are essential sources in the body. Iron (Fe) and vitamin C are factors associated with the formation of red blood cells and hemoglobin in the blood [28].

The average number of leukocytes, lymphocytes from day 0 and day 31 on males and females were decreased but were still within normal limits. This was possibly because of the content of anthocyanin and vitamin C from rosella which acts as an

antioxidant The role of anthocyanin and vitamin C as an antioxidant was binding free radicals and molecules that are highly reactive. High consumption of antioxidants could increase immune system against foreign objects or antigens [29]. Leukocytes play a role in natural immunity as phagocytes, antigens, and antibodies [30]. The number of leukocytes plays an important role in improving the immune system and also protects the body cells against pathogens [31]. Anthocyanin also plays a role in inhibiting the proliferation of lymphocytes. This proliferation of lymphocytes is associated with a process of haematopoiesis in the mechanism of apoptosis. The process of apoptosis plays an important role in maintaining the correct number of haematopoietic progenitors for erythrocytes as well as various types of leukocytes, such as lymphocytes. Each cell has a different lifetime. Lymphocytes have a life span of one day (neutrophil) and some are up to 20–30 years old for some T cells [32]. The circulation of lymphocytes in the blood increases, if the immune system increases compared to the state of illness, where the number of lymphocytes becomes indicator of immune response [33]. The comparison of haemoglobin, leukocyte, and lymphocyte levels on 31st and 45th days of both men and women had no significant difference ($p>0.05$). It indicated that delay of effect did not occur. The mean of male haemoglobin from day 31 to day 45 had increased, while in women, it decreased but was still in normal limit.

The comparison of haemoglobin and leukocyte levels on days 0 and 45 days was not significant ($p>0.05$), while the percentage of lymphocyte revealed the significant difference ($p<0.05$). However, the lymphocyte percentage during the study was within the normal range of 15–45% [34].

2 The effect of ethanolic extract of rosella on BUN

Table 2 and Table 3 revealed that BUN levels of male and female volunteers in the day 1 had no significant difference compared to those of day 31 ($p>0.05$). However, there was a significant difference in the average BUN level from each measurement day. A decrease in BUN levels from day 0 and 31, however these values are within normal levels.

On the day 31 and 45 after not being given extract capsule, there was no significant difference ($p>0.05$). The use of rosella extract on male volunteers decreased the amount of BUN and increased again on the day 45 (15 days after did not consume the rosella). In female volunteers, the number of BUN levels

remained at the same levels either on day 30 or day 45. The increased levels of BUN in women was in line with those reported by Ukoha *et al.* [35], indicating that the increased BUN is due to an integrity distortion of renal cytoarchitecture in the administration of rosella flower water extract for 21 days. It is because rosella has a diuretic effect, caused by quercetin and anthocyanin [36]. BUN levels are also influenced by the amount of protein intake in the body. The status of hydration is also a factor affecting the amount of BUN in blood [3].

SGOT and SGPT

The comparison of SGOT and SGPT levels was shown in table 2. There is no significant difference ($p>0.05$) between day 0 and day 31 in male and female volunteers. The average of SGOT and SGPT on days 0 and 31 decreased in males but did not in females. Clinically, the average levels in days 0 and 30 were still within normal limits.

The comparison of SGOT and SGPT levels in day 30 and day 45 of male and female shows no significant difference ($p>0.05$). The mean of SGOT in males decreased while in females remained at the same level, while SGPT levels decreased both in females and males. This decrease in value is in line with previous studies showing that ethanol extract of red rosella flower petals has a hepatoprotective activity by decreasing the activity of SGOT and SGPT [14].

CONCLUSION

The administration of rosella extract capsule for 30 days did not affect the vital sign, haemoglobin value, leucocytes, lymphocytes, BUN, SGOT, and SGPT ($p>0.05$). In addition, the ethanolic extract of rosella does not negatively affect the vital signs, haemoglobin, leukocytes, lymphocytes, BUN, SGOT, and SGPT, thus, its consumption is safe.

ACKNOWLEDGEMENT

The leading research grant of study program from Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPP) Universitas Ahmad Dahlan (UAD) in 2016 and 2017 PUPS-049/LPP-UAD/III/2016; PHB-212/LPP-UAD/III/2016; PIPP-001/SP3/LPP-UAD/IV/2017.

Conflict of interest: Authors declare no conflict of interest.

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