Adaptogenic effect of the ethanol extract of mangosteen peel (Garcinia mangostana L.) in mice (Mus musculus) blood profile using the Swimming Endurance Test

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Abstract. The adaptogenic activity was related to antioxidant activity, which can reduce the number of free radicals, and in turn, will reduce stress and improve body balance. Mangosteen rind (Garcinia mangostana L.) with a large antioxidant content promises to be Adaptogen. This study was aimed to examine the Adaptogenic effects of ethanol extract of mangosteen peel (Garcinia mangostana L.) Using the Swimming Endurance Test (SET) in mice. Simplicia of mangosteen Fruit (Garcinia mangostana L.) extracted by maceration using 96% ethanol, then tested for its Adaptogenic activity based on swimming endurance test and routine serum blood profile of mice including blood glucose, RBC and WBC count in five treatment groups. The groups consisted of negative control of NaCMC 1%, positive control of Vit C. 100 mg/kg BW and three groups treatment of ethanol extract of mangosteen peel (Garcinia mangostana L.) ranging from 100, 200 and 400 mg/kg BW. All groups were given treatments once daily per-oral for seven days, further on the 8th day, a SET test and blood serum profile test were performed. The results showed that the ethanol extract of mangosteen peel (Garcinia mangostana L.) had the ability to be Adaptogenic. The RBC count and WBC count showed normal value after stress induction by SET. The adaptogenic of mangosteen peel extract reduced the increase in blood glucose level after mice experienced significant stress.

1. Introduction

Stress is caused by a stressor which affects the body's homeostatic system [1] and also by the formation of radicals which cause oxidation of nucleic acids and proteins, damage the bio-membrane and trigger lipid peroxidation [2]. Free radicals in the body can be neutralized with a compound, antioxidant [3]. Stress not only afflicts humans but also animals, especially pets, with the symptom of hair loss.

Adaptogens cause adaptive reactions which help the body to adapt to stressful conditions to maintain the body homeostatic system. Adaptogenic activity is related to the antioxidant activity of a plant [4]. Under stressful conditions, activation of proteins or enzymes such as JNK can result in an increase in NO free radical formation. Molecular mechanism of adaptogens through inhibition of NO radicals restore ATP production and prevent protein damage [5]. The mangosteen peel (Garcinia mangostana L.) contains active compounds including flavonoids, tannins and xanton [6] and has a
large antioxidant activity with IC$_{50}$ of 44.49 mg/L. The lower IC$_{50}$ value, the more activity of DPPH free radical deterrent [7]. This study was aimed to examine the Adaptogenic effects of mangosteen peel (Garcinia mangostana L.) ethanol extract in mice using the Swimming Endurance Test.

2. Methods

2.1. Mangosteen peel extraction

Simplicia powder produced from the drying and grinding process of mangosteen fruit weighed as much as 3 kg, then macerated with 7.5 L of 96% ethanol solvent for ± 4–5×24 hours with occasional stirring and followed by filtering. The residue was re-macerated with 5 L ethanol. Re-maceration was done once for ± 3×24 hours. The filtrate then collected and evaporated using a rotary evaporator at 45–50°C to obtain the thick extract with the modified method [3], to produce mangosteen peel extract. Further, phytochemistry test was conducted to determine the flavonoids, alkaloids, tannins, polyphenols, and saponins content qualitatively [8].

2.2. Experimental animals and treatments

The experimental animals used for SET were 25 male mice with 25–30 grams of BW which divided into five treatment groups as follow: Group 1 as negative control given 1% CMC-Na; group 2 as positive control given 100 mg/kg BW vitamin C; group 3, 4, and 5 given 100, 200, and 400 mg/kg BW of mangosteen peel extract, respectively [7]. Acclimatization period was seven days. The mangosteen peel extract was administered once daily for seven days.

2.3. Swimming endurance test (SET)

Swimming endurance test (SET) was a screening model for antidepressants and or adaptogens [2] and adapted from [3]. This method was carried out by observing experimental animals which forced to swim in the water until they experienced an immobile state indicated fatigue condition [9]. At 8th-day SET was done using glass vessels with a height of 45 cm and a diameter of 22 cm, filled with water at room temperature up to a height of 20 cm [10]. Mice were allowed to swim until they experienced an immobile state [3]. Blood samples were collected at the 8th day through a retro-orbital puncture for further RBC count, WBC count, and serum glucose determination.

3. Results and discussions

3.1. Phytochemistry screening

Phytochemistry screening of mangosteen peel extract showed negative content of saponins (table 1).

| Phytochemistry content | Result |
|------------------------|--------|
| Alcaloids              | positive |
| Flavanoids             | positive |
| Tannins                | positive |
| Phenolics              | positive |
| Saponins               | negative |

Qualitative phytochemistry screening of mangosteen peel extract showed alkaloids, flavonoids, phenols, and tannins but none saponin content. A study conducted by [8] using mangosteen fruit showed saponin content from the ethanol extracted mangosteen peel. Mangosteen fruits in this study originated from Sabang sub-district, North Luwu Regency, South Sulawesi province, while in the
previous study, mangosteen fruits were obtained from Bali. The different location might cause a difference in secondary metabolite content since the soil elements were not homogenous.

3.2. RBC Count, WBC Count, and serum glucose content
The blood profile for RBC count, WBC count, and glucose content in all groups (table 2) showed a normal range (standard value) according to [11].

| Treatment Group | RBC (×10^6/mm³) | WBC (×10^3/mm³) | Glucose (mg/dL) |
|-----------------|-----------------|-----------------|-----------------|
| Negative control 1% CMC-Na | 8.3 | 2.3 | 110 |
| Positive control 100 mg/kg BW vitamin C | 7.9 | 3.1 | 102 |
| 100mg/KgBW Mangosteen peel extract | 7.4 | 2.8 | 105 |
| 200mg/KgBW Mangosteen peel extract | 8.1 | 3.2 | 92 |
| 400mg/KgBW Mangosteen peel extract | 8.2 | 2.7 | 87 |
| Standard value* | 7.1–9.5 | 1.5–4.8 | 75–128 |

*reference value according to [11]

The stress conditions caused by the SET Test would increase blood biochemical parameters such as RBC, WBC, Glucose, and cholesterol [10]. Stress will stimulate the increase in plasma Cortisol value in Humans, which in rodents is corticosterone [12]. Increasing levels of cortisol and corticosterone will cause the mobilization of carbohydrates and fats, as of blood glucose and cholesterol will increase. Table 2 showed that all treatment groups were in the normal range of RBC count, WBC count, and blood glucose level. However, the blood glucose level was highest in the negative control group without administration of vitamin C and mangosteen peel extract while the lowest was in the treatment group given 400 mg/kg BW mangosteen peel extract. The adaptogenic of mangosteen peel extract reduced the increase in blood glucose level after mice experienced significant stress.

4. Conclusion
Ethanol extract of mangosteen peel (Garcinia mangostana L.) showed antistress adaptogenic effects in mice with the decreases in RBC count, WBC count, and blood glucose level, after mice experienced stress stimulation.

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