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**Abstract.** *Garcinia mangostana* L. (mangosteen) pericarp containing α-mangostin is predicted to have potential as an anticancer because it can induce apoptosis and has high antioxidant content. Moreover, the preliminary study showed that this extract has high antioxidant activity and cytotoxicity based on the brine shrimp lethality test. This indicates that mangosteen has potential as chemotherapeutic agents or additional therapy in cancers such as colorectal cancer, which the available management is not as effective. To improve its efficacy and bioavailability in the colon as a targeted area, ethyl acetate fraction of mangosteen extract was then formulated into a controlled release encapsulated microparticles by chitosan-alginate material. It is intended that the active ingredient to be released after reaching the colon. In this study, toxicity of mangosteen was investigated because there is still no toxicity data of encapsulated microparticles of the ethyl acetate fraction of mangosteen extract at a single dose of 2 and 3 g/kg BW, however, significant increases of BUN and SGOT levels were found in single dose of 5 g/kg BW. The results indicate that administration encapsulated of microparticles ethyl acetate fraction of *G. mangostana* L. extract at single dose of 2 and 3 g/kgBW cause no toxicity to the liver and kidneys, but the single dose of 5 g/kgBW cause toxicity to the liver and kidneys. Further studies on histopathology examinations of liver and kidneys are necessary to ensure its safety level.

Keywords: acute toxicity, control released, ethyl acetate fractionation, *Garcinia mangostana* L., microencapsulation

**INTRODUCTION**

Colorectal cancer is a cancer that is formed from the tissue of colon which consists of colon and or rectum [1]. In Indonesia, colorectal cancer is ranked the third cancer with estimated mortality rate 9.5% among other cancer cases [2-5]. The risk factors of colorectal cancer are genetic, limited activities, obesity, low fiber and high fat diet, low vitamin D, smoking, alcohol consumption, and drugs [1,6,7]. With the westernization in Indonesia that affect life style and diet, the incidence of colorectal cancer in Indonesia has increased to 12.8 per 100.000 [6-9]. However, the
treatment of choice, surgery, is usually not affordable and gives many side effects. Other treatment options for colorectal cancer are chemotherapy and radiotherapy [5]. However, these therapies have some major side effects and limitations for Indonesia, and the costs are also high.

Knowing the limitations of these treatments, alternative treatment that costs less and has effective effects for colorectal cancer is needed. One of the potential substances is from a fruit plant, *Garcinia mangostana* L. Many studies found that the extract of *G. mangostana* L. is beneficial as an anticancer agent for cancers, including colorectal cancer [10-15]. As colorectal cancer is located in the gastrointestinal tract, the medicine should be encapsulated with polymer substances so the drugs can be slowly released in the colon. Chitosan-alginate encapsulation microparticles are proven to be the basic formulation for controlled drug release [11].

In this research, the extract of *G. mangostana* L. which is encapsulated by chitosan-alginate microparticles was given to 4 groups of BALB/c mice to find the oral acute toxicity of the drug with different doses on marker function of mice’s kidneys using BUN (Blood Urea Nitrogen) and serum creatinine and liver using SGOT (serum glutamic oxaloacetic transaminase) and SGPT (serum glutamic pyruvate transaminase). The objectives are to evaluate the effect of this drug to SGOT, SGPT, BUN, and creatinine serum levels so it can be used as a consideration for a safe anti-colon cancer agent. Elevation of marker levels of function kidneys and liver indicate toxicity and reduction of their function.

**MATERIALS AND METHODS**

**Plant material and extract preparation**

The extract of chitosan-alginate microparticles encapsulated mangosteen was obtained as a result of a research that was done by Faculty of Engineering, Universitas Indonesia. The pericarp of mangosteen was obtained in 2014 from Solo and was labeled as *Garcinia mangostana* Linn. by Herbarium Bogoriense, Research Center for Biotechnology-Indonesian Institute of Sciences (LIPI). Standard α-mangostin (98%) was from China and the chitosan (medical grade; deacetylation degree of 93.6%; viscosity of 23.3 cp) was obtained from Biotech Surindo, Indonesia. Other materials such as calcium chloride and sodium alginate were obtained from Merck. While Sodium tripolyphosphate (food grade) was obtained from Brataco Chemical, Indonesia. The extract that was already within ethyl acetate fraction was then suspended in Department of Medical Pharmacy, Faculty of Medicine Universitas Indonesia.

The dry pericarp of mangosteen was extracted using a modified procedure stated by Jung, *et al* [12]. Subsequently, the extract was macerated using ethanol 96% for seven days applying mangosteen powder to ethanol ratio of 1:3 (m/v) and periodic stirring was done to the mixture. The macerated mixture was then filtrated and evaporated under a reduced pressure to be crude ethanolic extract. Afterwards, the mixture was fractionated using a mixture of ethyl acetate and water with 1:1 volume ratio. The evaporation of this ethyl acetate fraction under reduced pressure generated mangostin powder/paste [11]. The mangostin powder was then emulsified using gum arabic to form a more soluble drug to be administrated.

**Experimental Animals**

Female BALB/c mice were obtained from Central of Health Research Development, Ministry of Health of the Republic of Indonesia and acclimatized to the laboratory conditions. Twenty 6-8 weeks, nulliparous and not pregnant mice, 25-30 g of body weight with 20% maximum of weight distribution were prepared. All mice were housed 5 per cage in a room maintained at 25°C, 50 ± 10% relative humidity and a 12-hour-light/12-hour-dark cycle throughout the experiment. All mice had free access to water and food before the beginning the experiment. All of the procedures in this study have been approved by the Health Research Ethic Committee Faculty of Medicine, Universitas Indonesia (Approval no. 0430/UN2F1/ETIK/2018).

**Acute Oral Toxicity Study and Dose Administration**

The mice were randomly divided into 4 groups (n=5) i.e. three experimental groups and one control group, with each group contains five mice [16]. The extract in doses of 2, 3 and 5 g/kgBW. Mice are fasted for 3-4 hours (except water) before treatment. The administration of mangosteen extract dissolved in a solvent (distilled water and gummi arabicum), and it was administered through intragastric in a single oral dose by gavages using a feeding needle with maximum volume 1 ml/100 g mice. The control group received an equal volume of solvent. The animals were then
weighed and given a single dose of mangosteen extract based on their body weight. In the circumstance where it is not possible to administer a dose with a single feeding, the extract of mangosteen can be administered several times within 24 hours. The feeds may be given 1-2 hours after treatment. The mice were maintained within 14 days observations. After administration, the mice were observed during the first day and each of the following 14 days to look for any signs and symptoms of toxicity and death. After it was done, all of the remaining mice were sacrificed to see the clinical biochemistry in their blood specimen.

**Biochemical Analysis**

After the 14 days observations, the remaining mice were anesthetized for blood collection. Blood samples were centrifuged and the supernatant were stored. Examination of clinical biochemistry including SGOT and SGPT levels are used to determine the liver function, while BUN and serum creatinine concentration levels are used to determine the kidney function. The blood samples was examined using Spectrophotometry.

**Statistical analysis**

All data was processed using Stat view 5.0 software. Statistical significance of the difference of clinical biochemistry in the four groups was tested using one-way analysis of variance (ANOVA). Significance of difference was considered at $p < 0.05$. The difference among groups was analyzed using Fisher’s Paired Least Significant Difference (PLSD) post hoc. If the $P$ value is less than 0.05, the result will be considered significant.

**RESULTS AND DISCUSSION**

<table>
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<tr>
<th>TABLE 1. The marker level of function of kidneys and liver after administration of the <em>G. mangostana</em> L. extract</th>
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<tbody>
<tr>
<td><strong>Dose administered (g/kgBW)</strong></td>
</tr>
<tr>
<td>Control</td>
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<td>3</td>
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*p < 0.05 vs. control group (0 g/kg BW)

Table 1 shows that all mice groups given single dose of mangosteen extract had higher BUN levels than the control group, but a significant increase occurred at the administration of 5 g/kgBW. Moreover, a significant increase of SGOT levels was found in the 5 g/kgBW group. On the other hand, serum creatinine and SGPT did not show any significant dose-related effects. This finding indicates that the administration of single dose encapsulated microparticles of the ethyl acetate fraction of mangosteen extract at 2 and 3 g/kgBW did not affect the marker of kidneys and liver function, but a single dose of 5 g/kgBW caused toxicity and reduction of the function of the liver and kidneys of mice. These findings were different from study by Kosem who conducted repeated administration of crude methanolic extract during a 14-day study showed renal and hepatic cell injury, resulting a significant increase of BUN in ≥500 mg/kg BW dose, SGPT in ≥250 mg/kg BW dose, and SGOT in ≥200 mg/kg BW dose.

**CONCLUSION**

From this research, it can be concluded that *G. mangostana* L. extract with ethyl acetate fraction encapsulated by chitosan-alginate microparticles is safe seen from kidneys and liver function at a dose until 3 g/kg BW. Therefore, this extract is potential to be a safe anti-colon cancer drug. However, further research on histopathological examination needs to be done to ensure the safety of this drug.
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