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Effect of Crude Ethanolic Extract of Mangosteen (*Garcinia mangostana* Linn.) on Intestinal SIgA and Bacterial Colonies in Intestine, Liver, and Spleen in Typhoid Mice Model

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Abstract : Typhoid fever, is a systemic infection caused by *Salmonella* enterica serotype Typhi (*S. typhi*) due to an invasion of intestine and other organs including liver and spleen by *S. typhimurium*, SIgA has been implicated in the removal of immune complexes, and in the neutralization of intracellular viruses Garcinia mangostana Linn is a tropical medicinal plant in Asia, its fruit pericarps, are nature's most abundant sources of xanthones, which help to maintain intestinal health and immunomodulation effect. This study used 24 mice divided into 6 groups, including control positive (infected with *S. typhimurium*), control negative (without infection), T0 (mice administrated with crude extract of mangosteen pericarp 40mg/ml), T1, T2 and T3 (treatment with 20 mg/ml, 40 mg/ml and 60 mg/ml of crude extract mangosteen pericarp). ELISA was used to measure sIgA level and culture was used to measure the bacterial colonies in intestine, liver and spleen. Result showed that the sIgA level increased significantly at the dose 40 mg/mL compared with C+ ($p<0.05$), but descriptively, the increased sIgA level started at dose 20mg/mL and getting low at higher dose 60 mg/mL. The bacteria colonies in C+ the significantly different to compare other group (C-,T0,T1,T2,T3) ($p>0.05$), the bacteria colonies start to decrease to zero in all treatment groups. In conclusion, the crude extract mangosteen pericarp can improve immune response by increasing the sIgA level and inhibiting the systemic diseases by prevent the bacterial pass from intestine and invading the organs (spleen and liver).
Keywords: Typhoid fever, mangosteen, S. typhimrium.

Introduction

Typhoid fever, a systemic disease caused by *Salmonella typhi* (*S. typhi*), remains an important public health problem in developing countries[1]. The bacteria is conscientious for estimated 16 million cases of systemic typhoid fever worldwide each year[2]. An infection of mice with *Salmonella typhimurium* (*S. typhimurium*) has consequences in systemic infection and a typhoid fever disease similar to that seen in humans after infection with *S. typhi*[3,4].

Salmonella enteric serovar *Typhimurium* is a genus of family Enterobacteriaceae and Gram-negative intracellular bacteria[5,6], *Salmonella* naturally infects a host by the oral route,and contaminated food. Infections with *Salmonella* enteric serovar *Typhimurium* (*S. typhimurium*) go to an acute intestinal inflammation in human

and animal hosts, as a result of the bacterium invading the mucosa. After oral infection, *Salmonellae* directly replicates in the mucosa-intestinal tissues (e.g. Peyer'spatches [PP]) and there after circulates via the mesenteric lymph nodes to systemic organs (e,g liver and spleen)[7,8]. The different stages of *S. typhimurium* infection are reflected in a variety of mechanisms of innate and acquired immunity which contributes to the response against this bacterium, included sIgA the important one of acquired immunity to response intestinal infection is mucosal immunity functional by sIgA ,To cover typhoid fever ,it's used antimicrobial agent ,but nowadays its found the bacteria to be resistant to many antimicrobial agents, So it makes necessary to fined compounds to make people healthy such as immunomodulator substances[9,10].

Garcinia mangostana Linn is nature's most abundant sources of xanthones in a class of polyphenolic compounds[11,12]. Those compounds are found in the pericarp, seed, bark, leaves, roots and flesh of mangosteen[13]. The crude ethanolic extract from mangosteen pericarp includes 25.19 % a-mangostin as an active xanthone[14]. Since xanthones have phenolic functional groups, they exhibit a wide range of biological activities including antimicrobial activities[15] and immunostimulant activity[16]. The extract also has confirmed antibacterial activities against a wide variety of microorganisms including *S. typhimurium*[17]. The polysaccharides and consumption of the micronutrient- and antioxidant obtained from mangosteen-fruit pericarp are found and can stimulate activities of phagocytic cells against *Salmonella* and regulate both the innate and acquired immune responses[16,18,19].

In this study it was used the ethanolic extract of mangosteen pericarp to observe its effect on improving the immune response by increasing the sIgA then the systemic disease is prevented when the *Salmonella* cannot penetrate the intestinal and invade the organ such as liver and spleen.

Materials and Methods

In this study 24 mice where divided into 6 groups consisting of 4mice, each namely the control positive (C+) infected with dose of 10^8 cfu bacteria *S. typhimurium* as control positive ,control negative (C-) without infection, T0 (without infection) which was administrated with 40 mg/ml CEMP(crud extract mangosteen pericarp) for 14 days as control treatment, group T1 which was administered with 20 mg/ml (CEMP) for 7days and following 7 days for *S. typhimurium* infection, group T2 which was administered with 40 mg/ml (CEMP) for 7days and following 7 days for *S. typhimurium* infection, group T3 which was administered with 60 mg/ml (CEMP) for 7 days and following 7 days for *S. typhimurium* infection. (Every mouse received 0.5cc/mice of concentration CEMP).

Infection preparation

After the last inoculation of the CEMP, mice were oraly infected with 300 µL *Salmonella typhimurium* bacteria (concentration $2x10^8$ cells/mice) two times with 2-days interval. Four days (96 hours) after the last infection, mice were sacrificed and t the small intestine, liver, and spleen where taken[20].

CEMP preparation

Pieces of Mangosteen pericarp where collected, dried and sieved to produce a fine powder, the powder was then weighed 100 grams each to be extracted by the macerated method and ethanol 96%. The extract was left to settle overnight. From 100 g dried pericarp powder it resulted the total extract 20-25 g.

sIgA examination procedures

Small intestine was opened and cleaned by remaining stool sterile PBS. Intestinal surface (intestinal mucus) was scraped by using 5 mL of PBS containing protease inhibitor cocktail (25 µg /mL). The intestinal mucus was homogenized by vortex for 1 minute then centrifuged at 12,000 rpm for 15 minutes at 4°C. Antibodies in the supernatant were purified by 40% ammonium sulfate. The mixture was centrifuged at 10,000 rpm for 15 minutes at 4°C. The precipitate was suspended with 1 mL of sterile PBS and dialyzed against sterile PBS. The dialysate (mucus sample) was stored and used for examination of secretory IgA levels (S-IgA). S-IgA examination performed by ELISA using anti-mouse IgA labeled with alkaline phosphatase (indirect ELISA)[20].

Calculate bacteria colonies in intestine and spleen

Intestine and spleen were washed with sterile PBS, then homogenized by Potter homogenizer. The liver and spleen homogenate (100 µL) were grown in BSA medium. Intestinal homogenates were diluted 10^{-3}, then 25 µL of it was grown in BSA medium. The Solid BSA medium was incubated for 18-24 hours at the temperature of 37°C. Colonies with characteristics of *S. typhimurium* were calculated by colony counter[20].

Statistical analysis

The data of this study were the number of bacteria on intestine while, liver and spleen were be analyzed by Kruskal Wallis and Man Wanity. The data of sIgA amount from mucosal intestinal were analyzed by one way ANOVA at the level of acceptance 95% (α=0.05).

Results

Effect of crude extract Mangosteen pericarp on levels sIgA mucous

The result (Figure. 1) showed the difference in the levels of sIgA was affected by doses of CEMP in mice infected with *S. typhimuriumin*. The result showed high levels of sIgA at 20 mg/ml dose (T1), 40 mg/ml dose (T2) and 60 mg/ml dose (T3), compared with sIgA levels in the positive control group which became higher after given CEMP. The administration of CEMP showed different influence of sIgA at 20 mg/mL dose (T1), 40 mg/mL dose (T2) compared with higher 60 mg/L dose (T3), (C+) control positive group and treatment control group (T0), C+ was getting down compared with (C-) control negative. Thus, based on the descriptive assessment according to the mean of sIgA levels, it can be said that administrating treatment in the form of Mangosteen extract at 20 mg/mLdose (T1), and 40 mg/mL dose (T2), showed different influences, where higher dose of provided Mangosteen extract further increased the sIgA levels. However,at of 60 mg/mLdose (T3), higher dose of CEMP on sIgA levels was getting down.

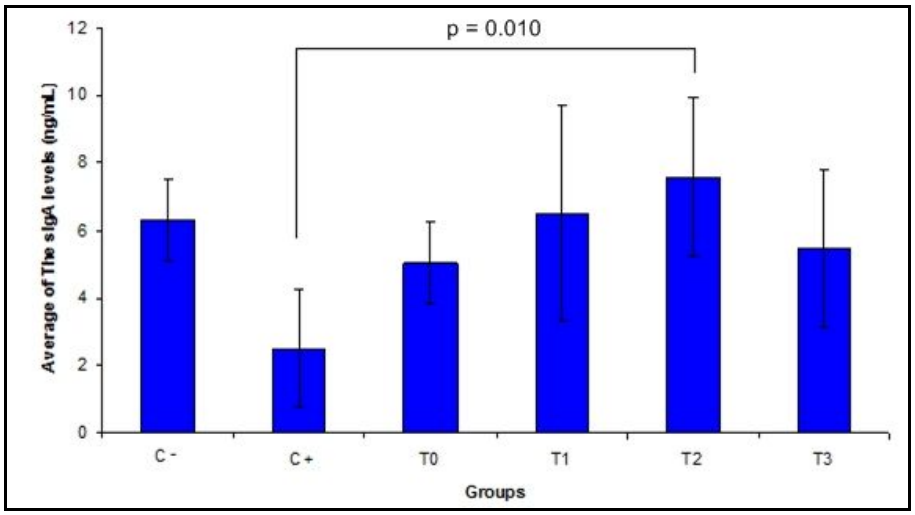

Figure 1. sIgA level after CEMP treatment on typhoid mice model
Notes:-

C-	**: negative control**	**C+**	**: positive control**
T0	**: control treatment**	**T1**	**: treatment group 1**
T2	**: treatment group 2**	**T3**	**: treatment group 3**

Effect of crude extract Mangosteen pericarps on amount of *S. typhimuriumin* colonies

The study showed (Figure 2) that the difference in CEMP dose give the same effects on *Salmonella* colonies in intestine, liver and spleen samples of mice. The effect of CEMP was observed that the *Salmonella* colonies of Intestine liver and spleen samples of the mice induced *S. typhimurium* bacteria in the positive control group become zero after the treatment was given in the form of CEMP started at 20 mg/mL dose (T1),

compared with *Salmonella* colonies in Intestine, liver and spleen samples of mice in the positive control group. The *Salmonella* colonies on intestine, liver and spleen samples of mice were still zero in higher doses. Thus, based on the descriptive assessment and the mean of *Salmonella* colonies in intestine, liver, and spleen samples of mice, it can be said that the CEMP administration at 20 mg/mL (T1), 40 mg/mL (T2), and 60 mg/mL (T3) doses showed the same effect compared with *Salmonella* colonies in Intestine, liver and spleen samples of mice in the positive control group ($p>0.05$).

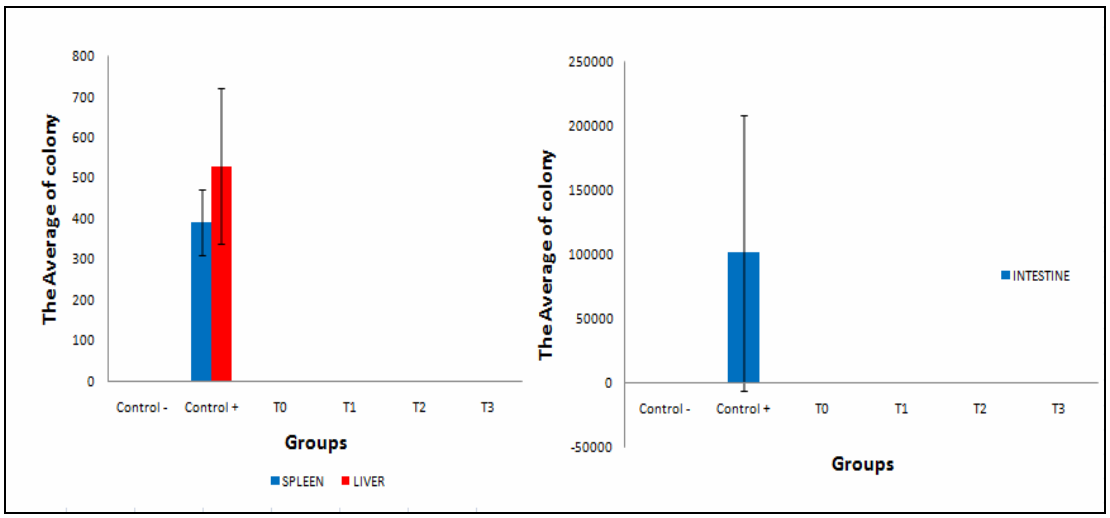

Figure 2. The amount of Salmonella colony on intestine, liver and spleen after treatment with Mangosteen extract

Note: The comparisons of the colony at Spleen, Liver and intestine between group C + and group C-, T0, T1, T2 and T3 are significant differences with p-value less than alpha 0.05. But the comparisons of the colony between group C-, T0, T1, T2 and T3 did not differ significantly.

Discussions

Effect of crude extract Mangosteen Pericarp on levels of mucous sIgA

Secretory IgA (SIgA) plays an important role in the protection and homeostatic regulation of intestine**.** This primary function of SIgA is referred to immune exclusion, a process which limits the access of numerous microorganisms and mucosal antigens to these thin and susceptible mucosal barriers[21]. SIgA production against specific mucosal antigens is dependent on the sampling by Peyer's patch M cells, processed by antigen-presenting cells such as dendritic cells (DCs), T-cell activation, and ultimately B-cell class switch recombination in gut-associated lymphoid tissues (GALTs), mesenteric lymph nodes, and possibly neighboring lamina propria[22].

Several cytokines, such as (IL)-4, transforming growth factor-β, IL-5, IL-6, and IL-10 are instrumental in intestinal stimulation of SIgA production. A subset of these cytokines, notably transform growth factor-β and IL-10[10]. A recent study showed the different effect of crude extract mangosteen pericarp on SIgA level in typhoid mice model, the best dose of mangosteen was 40mg/kg because it resulted ($p>0.05$) an increase of SIgA levels compared whit the control positive group. Higher dose (T3= 60mg/ml) decreased the SIgA levels. Hence, 40mg/mL dose was more effective to increase the SIgA level than 60mg/ml dose, however, 40mg/ml dose in treatment group without infection had ability to increase the SIgA level when compared with control positive group. The level of SIgA in control negative was high because its general antibody was not specific for *S. typhimurium*. the levels of SIgA in control negative was going to decrease it probably due to the effect of *salmonella* which could inhibit the DC (Dendritic cells) as an important APCs linking innate and adaptive immunity and *Salmonella* affecting the capacity of DC to present Ags in which their ability can stimulate T cell proliferation and not activate B cell to produce SIgA[23]. The dose of 20mg/ml showed more induction to the SIgA levels than T0 (treatment control) because the CEMP might induce the acquired immunity such as SIgA in a normal range. *Salmonella* also induces the SIgA secretion through the B cells activation[24,19]. The dose of

40mg/ml was also observable and showed higher increase of SIgA levels, but it tended to decrease at higher concentrations (60mg/mL).

The increase of medication dose should increase the response proportionally to the increased dose, but in this study it showed that the increase of concentration, followed the decreased response ultimately. This is because an overdose cannot afford to raise the rate to respond more[25]. This usually happens in natural medicines, chiefly in the use of extracts, because the extract does not contain a single compound but consists of various chemical compounds, in which these components work together to cause an effect. If the extract dose increases, the number of contained chemical compounds will increase, resulting in adverse interactions that lead to a decrease in effect[13]. The something was also observed in the effect of CEMP in mice's blood glucose levels giving by doses 50,100, and 200 mg/kg of body weight . The study showed the decrease of blood glucose levels was more effective at dose 100 mg/kg of body weight compared to the higher dose 200 mg/kg of body weight[26].

The fruit pericarps, which are rich sources of xanthones, are natural chemical substances possessing numerous bioactive properties which help to maintain intestinal health, to neutralize free-radicals, help functions and promote immunomodulation systems[12]. There are studies indicate that the intake of antioxidant-rich product from mangosteen significantly enhances immune responses and improves health status[19].

In this study, the results showed that mangosteen increased the SIgA levels at dose 40mg/ml, and stimulated the immune system through SIgA secretion.

Effect of crude extract Mangosteen pericarps on amount of bacterial colonies in intestine, liver and spleen

Salmonella typhimurium is a facultative intracellular pathogen which causes typhoid fever in humans (the only known natural hosts and reservoir of infection). Typhoid is a systemic disease that its bacteremic phase of disease is characterized by the dissemination of organisms. The most commonsites of secondary infection are the liver, spleen by penetrating the bacteria from intestine to organs such as spleen and liver and make colonies[27].

Our result showed the ability of mangoseen pericarp extract to prevent the systemic disease in mice infected with *S. typhimurium*, It appeared in all groups of CEMP treatment (T1 20mg/ml, T2 40mg/ml and T3 60mg/ml) in intestine spleen and liver, compared with control positive group. Thus, we could say the CEMP could prevent the bacteria to penetrate the intestinal when invading the other organs. Mangosteen may effect directly on bacteria to kill it or may It help the immune system to protect itself from the invasion of bacteria by activating immune cells to secrete cytokines and interleukins both of which help to stimulate the immune system for body protection from bacteria[28].

Base on the result above, it was concluded that Mangosteen pericarp have an ability to improve the immune system through increase the secretion of intestinal SIgA and can prevent the bacterial invasion into organs such as liver and spleen.

Conclusion

The effect of CEMP at dose 40 mg/ml has a significant effect to induce sIgA levels secretion, and the activity of CEMP significantly decreases the *S. typhimurium* colonies in intestine, liver and spleen on typhoid fever mice model.

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