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Original paper

Achras Zapota L Extract Reduces Levels of Soluble Tumor Necrosis Alpha (TNF-α) in *Salmonella typhi* Infected Animals

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Abstract

Background and aim: The benefits of manila sapodilla fruit have been widely known empirically in the community, namely raw fruit used for the treatment of typhoid fever. This study is to prove the effect of Manila fruit in reducing levels of tumor necrosis factor alpha (TNF-α).

Materials and methods: The design of this study uses a randomized design with Matching Pre test - Post test Comparison Group Design. The animals were divided into negative control group (induced by *S. thypi* without therapy), and groups treated with 510, and 750 mg/ kg BB of *Manila sapodilla* (*Achras Zapota* L) extract, and positive control group treated with Levofloxacin 98 mg / kg BB. Serum TNF-α level was assayed in 4, 10 and 30 days after treatment.

Results: A significant decrease in TNF-α concentration was found ($p<0.05$) in all experimental groups at observation day 4, 10 until the 30th day after treatment.

Conclusion: Treatment with Manila *sapodilla* fruit extract (510 and 750 mg / KgBB) can reduce levels of soluble in TNF-α in *Salmonella typhi* infected animals.

Keywords: *Achras zapota L, TNF-α, Salmonella typhi*

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Introduction

Sawo manila (*Achras zapota* L) is a fruit tree that can bear fruit throughout the year. Sapodilla manila has a large and shady tree, can grow to as high as 30-40 m. The single flower is located in the armpit of the leaf near the tip of the twigs, stemmed from 1-2 cm, often hanging, the flower diameter up to 1.5 cm, the outer side is brownish-haired. Petals are usually arranged in two circles; crown, clapper shape, white, sharing up to half the length of the tube. Single leaves, located alternately, often collect at the end of the branch. Flat-edged leaves, slightly hairy, shiny dark green, round-shaped shape jorong to slightly lanceolate, 1.5-7 x 3.5-15 cm, base and ends of the shape of a wedge, stemmed with 1-3.5 cm, leaf bones main protruding on the lower side. Low branching, brown manila stem with rough skin blackish gray to dark brown. All parts contain latex thick milky white sap [1],[2],[3].

Benefits of sapodilla fruit have been widely known empirically in the community, namely raw fruit is used for the treatment of typhoid fever by raw fruit in washing / cleaning then the fruit is shredded and the results of grater squeeze using fine leaves and the filter is drunk in patients with typhoid fever [4]. Ripe fruit can be used as ingredients for making syrup or if fermentation can be made into wine or vinegar. The tree itself can be an ornamental plant or medicinal plant that can be utilized by the community [5],[6], [7].

Typhoid fever is a disease caused by gram-negative bacilli bacteria, known as *Salmonella typhi* where these bacteria enter through contaminated food. The animal vector that carries these bacteria is cockroach legs, fly legs and mouse feet. After entering the human digestive tract, this bacterium then enters and multiplies in the intestine and spreads throughout the bloodstream, causing fever [8],[9].

The first antibiotic to treat typhoid fever was chloramphenicol, used in 1948 and subsequently became the preferred therapy for up to three decades in addition to ampicillin and trimethoprimsulfamethoxazole. The first report on the resistance of Salmonella typhi to chloramphenicol in 1974, twenty years later reported resistance of *Salmonella typhi* to chloramphenicol, ampicillin, and trimethoprimsulfamethoxazole, otherwise known as MDR (multiple drug resistance) *Salmonella typhi*. At present the increase in *Salmonella typhi* resistance to second-line therapy, 3rd generation cephalosporins and quinolone groups has also been widely reported [10],[11].

TNF-α plays a role in host defense for bacterial, viral and parasitic infections. TNF-α is produced by macrophages and is activated by T cell lymphocytes, antigens, NK cells, and mast cells. TNF-α is usually not detected in healthy individuals but is often found in conditions of inflammation and infection in the serum. TNF-α works against leukocytes and endothelium, induces acute inflammation at low levels9 because TNF-α is a strong pyrogen [12]. TNF-α plays a role in systemic inflammation at moderate levels. TNF-α causes pathological abnormalities in high levels of septic shock, because TNF-α is cytotoxic [13].

Materials and Methods

This research is purely experimental (True-Experimental Design) using a Completely Randomized Design (CRD). The design of this study uses a randomized design with Matching Pre test - Post test Comparison Group Design.

Manila sapodilla fruit

Manila Sapodilla fruit was selected and cleaned.

Experimental animals

Balb / c mice (aged 8-12 weeks, weighing 30-40 grams; n = 25) were maintained in the

Molecular Biology and Immunology Laboratory, Microbiology Department Faculty of Medicine, Hasanuddin University (Makassar, Indonesia). The mice were acclimatized for 8 days and intraperitoneally injected with *S. Typhi* strains thy1 (3 ml x 103 ml / CFU). The animals were divided into four treatment groups (5 mice in each group): negative control animals (Placebo) which treated with placebo, animals treated with 510 mg / kg BB of *Manila sapodilla* extract (ESBM 510), animals treated with 750 mg / kg BB of *Manila sapodilla* extract (ESBM 750) and positive control group treated with 98 mg/kg BB of Levofloxacin. TNF-α serum levels were measured using human TNF-α Enzyme-Linked Immunosorbent Assay (ELISA) test kit.

Levofloxacin

Levofloxacin was obtained from Kimia Farma, Pharmaceutical, Indonesia and was dissolved in distilled water and given through the nasogastric sonde, once a day for five days.

Sampling of peritoneal fluid and bacterial colony examination

Mice were fixed in the supine position, the abdomen is cleaned with alcohol 70% and 0.8-1 mL saline was injected into the peritoneal cavity. Mice were then allowed to stand for 1 minute. Peritoneal fluid removed from the peritoneal cavity of mice, then fluid aspirated by 0.5 mL syringe. Peritoneal fluid retrieval was performed three times (4, 10 and 30 days after *S. typhi* injection).

Bacterial colonization was examined by using the pour plate method. This method was performed by diluting the peritoneal fluid samples of 0.5 mL in 4.5 mL of saline (0.9% NaCl). Approximately 1 mL of the suspension was poured into a sterile petri dish, followed by pouring the fertilizer medium (nutrient agar) then sealed and incubated for 1-2 days at a temperature of 37^0C.

Statistical analysis

Data are expressed as the mean ± SE. To assess differences in levels of VDR and bacterial colony count between groups, paired t- test was used with the SPSS 23 software. A p-value less than 0.05 was statistically significant.

Ethics statement

This study was approved by the Health Medical Research Ethics Committee at the Faculty of Medicine, Hasanuddin University (Makassar, Indonesia) with registration number 902 / H4.8.4.5.31 / PP36-KOMETIK / 2018 on October 31, 2018.

Results

To determine the effects of sapodilla ekstract on reduce of soluble Tumor Necrosis alpha (TNF-α) used paired T test to assess the dynamics of in the observation time for each group. Summary results can be seen in Table 1.

Table 1. Serum TNF-α levels in positive control, negative control, and treatment groups.

Groups	Serum TNF-α level								
	Pre (H-4)	Post (H-10)	p	Pre(H4)	Post (H-30)	p	Post (H-10)	Post (H-30)	p
	mean±SD	mean±SD		mean±SD	mean±SD		mean±SD	mean±SD	
EBSM 510	190.77±13.23	178.65±18.03	0.011	190.77±13.23	157.44±27.29	0.024	178.65±18.03	157.44±27.29	0.052
EBSM 750	217.70±22.19	197.84±24.12	0.004	217.70±22.19	145.32±28.93	0.018	197.84±24.12	145.32±28.93	0.064
Levofloxacin	306.57±20.67	290.69±22.31	0.023	306.57±20.67	156.80±17.97	0.001	290.69±22.31	156.80±17.97	0.001
Placebo	188.92±10.75	179.90±12.82	0.003	188.92±10.75	159.33±21.41	0.012	179.90±12.82	159.33±21.41	0.032

Values are mean± SD of n = 5, P-value of <0.05 is significant. Baseline (H0) before *Salmonella typhi* infection; Pre (H4) after *Salmonella typhi* infection; Post (H10) after intervention of Manila extract; (H30) maintenance after intervention.

Table 1 shows that a significant decrease in TNF-α concentration was found (p = 0.011, p <0.05) in the group given EBSM 510 mg / KgBB in observation (H-4) to (H-10); The same thing happened in the 750 mg / KgBB EBSM group which found a significant decrease in TNF-α concentration (p = 0.004, p <0.05); In the Levofloxacin 98 mg / KgBB group it was also found a significant decrease in TNF-α concentration (p = 0.023, p <0.05); The same thing also happened in the aquades group, which found a significant decrease in TNF-α concentration (p = 0.003, p <0.05).

Observations (H-4) to (H-30) found a significant decrease in TNF-α concentration in the four groups, EBSM 510 mg / KgBB (p = 0.024, p <0.05), EBSM 750 mg / KgBB (p = 0.018, p <0.05), Levofloxacin 98 mg / KgBB (p = 0.001, p <0.05) and aquades (p = 0.012, p <0.05). Whereas for observation (H-10) to (H-30) there was no significant decrease in TNF-α concentration in the group namely EBSM 510 mg / KgBB (p = 0.052, p> 0.05) and EBSM 750 mg / KgBB (p = 0.064 , p> 0.05), different from what happened in the Levofloxacin group 98 mg / KgBB (p = 0.001, p <0.05) and aquades (p = 0.032, p <0.05) found a significant decrease in TNF-α concentration.

Table 2. Differences in changes that occurred between Pre (H-4) observation groups after injection of *Salmonella typhi*, post (H-10) after administration of *Manila sapodilla* fruit extract and post (H-30) maintenance after the preparation of *Manila sapodilla* fruit extract.

Groups		**Serum TNF-α level**			
		Pre (H-4)	Post (H-10)	Post (H-30)	p
EBSM 510	Mean	190.77	178.65	157.44	0.024*
	SD	13.23	18.03	27.29	
EBSM 750	Mean	217.7	197.84	145.32	0.018*
	SD	22.19	24.12	28.93	
Levofloxacin	Mean	306.57	290.69	156.8	0.001*
	SD	20.67	22.31	17.97	
Placebo	Mean	188.92	179.9	159.33	0.012*
	SD	10.75	12.82	21.41	

Values are mean± SD n = 5, P-value of <0.05 is significant. ; ESBM (Sapodilla fruit extract). Baseline (H0) before *Salmonella typhi* infection; Pre (H4) after *Salmonella typhi* infection; Post (H10) after intervention of Manila extract; (H30) maintenance after intervention.

Table 2 shows that a significant decrease in TNF-α concentration was found (p = 0.024, p <0.05) in the group given EBSM 510 mg / KgBB in observation (H-4) to (H-10) to (H-30); The same was found in the group given 750 mg / KgBB EBSM which found a significant decrease in TNF-α concentration (p = 0.018, p <0.05); the group given Levofloxacin 98 mg / KgBB also experienced a significant decrease in TNF-α concentration (p = 0.001, p <0.05); the group given aquades also experienced a significant decrease in TNF-α concentration (p = 0.012, p <0.05). It can be concluded that there was a significant decrease in TNF-α concentration in all four groups.

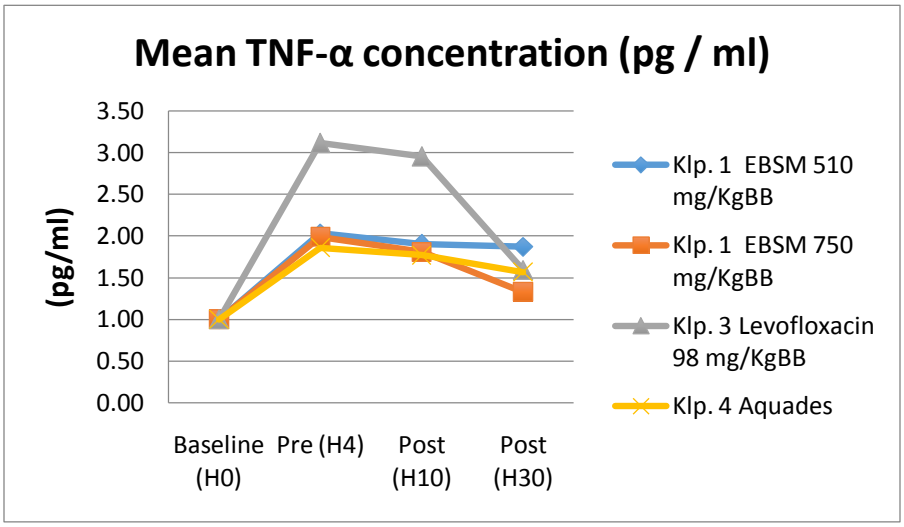

Fig. 1. Dynamics of change in TNF-α concentration by group during observation time; ESBM (Sapodilla fruit extract); Baseline (H0) before *Salmonella typhi* infection; Pre (H4) after *Salmonella typhi* infection; Post (H10) after intervention of Manila extract; (H30) maintenance after intervention.

Figure 1 shows that there was an increase in TNF-α concentration in all four groups after injection of Salmonella typhi bacteria by intraperitonium from (H-0) to (H-4). The highest increase in TNF-α concentration occurred in the Levofloxacin group 98 mg / KgBB (208.23 pg / ml), then followed by the EBSM group 750 mg / KgBB (108.44 pg / ml), then the EBSM group 510 mg/ KgBB (96.96 pg / ml) and the lowest increase in TNF-α concentration in the aquades group (87.33 pg / ml).

After intervention on (H-4), there was a gradual decrease in TNF-α concentration from (H-4) to (H-10) to (H-30) where the highest decrease occurred in the Levofloxacin group 98 mg / KgBB (149.77 pg / ml), followed by the EBSM group 750 mg / KgBB (72.38 pg / ml), then the aquades group (29.59 pg / ml), and the lowest decrease in TNF-α concentration occurred in the EBSM 510 mg / KgBB group (15.33 pg / ml). This means that EBSM 750 mg / KgBB can reduce TNF-α concentration not inferior to Levofloxacin 98 mg / KgBB with only a difference (72.38 pg / ml).

Discussion

Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine which plays a role in the inflammatory process, initiates polymorphonuclear (PMN) and activates it so that PMN can reach the site of infection [14]. Tumor necrosis factor alpha (TNF-α) is the main cytokine in the acute inflammatory response to Gram negative bacteria and other microbes. The infection process can trigger the production of TNF-α which can cause a systemic reaction. The main sources of TNF-α are mononuclear phagocytes and T cells that are activated by antigens, NK cells, and mast cells. Lipopolysaccharide is a patent stimulation of macrophages to secrete TNF-α [15],[16],[17].

Based on the results of our study in table 1, it was shown that after administration of sapodilla manila fruit extract dose of 510 mg / KgBB ($p = 0.011$, $p<0.05$) and 750 mg / KgBB ($p = 0.004$, $p<0.05$) a decrease in TNF-α concentration in mice infected with Salmonella typhi by intraperitonium. The same was found in Levofloxacin positive control 98 mg / KgBB ($p = 0.023$, $p <0.05$). This is due to the giving of brown manila fruit extract can reduce the amount of

Salmonella typhi bacteria so that suppressing the occurrence of direct infection in the host causes reduced release of cytokines by magrofags, one of which is TNF-α. This is also explained in a study conducted by Kema, (2016) where the infection of dengue virus causes plasma enlargement by endothelial activation, so that infected magrophages will become active and release cytokines including tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), IL-6, and platelet activating factor (PAF). So that by providing interventions that can reduce the number of bacteria that cause infection, it will also directly control the infection process and reduce the number of active cytokines due to inflammation [18].

The study conducted by Ali, (2013) found that by suppressing the concentration of tumor necrosis factor alpha (TNF-α) can protect the lungs from injury, this is due to an increase in excessive proinflammatory cytokines, one of which TNF-α is a major contributor to lung injury I. Research conducted by Rajakumar, (2011) also found that it was effective as an antibacterial in ten gram positive and twelve gram negative. This is caused by the content of compounds contained in brown manila fruit extract, namely tannin, flavonoids and triterpenoids which can inhibit bacterial growth and cause lysis bacteria [19].

Conclusion

Treatment with *Manila sapodilla* (*Achras Zapota* L) fruit extract (510 and 750 mg / KgBB) can reduce levels of soluble in TNF-α in *Salmonella typhi* infected animals playing a significant possible role in decreasing of inflammatory responses to *Salmonella typhi* infection.

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Conflict of interests

Authors declare that there is no conflict of interests within this research article and publication.

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