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Mangosteen Extract Reduces the Expression of Matrix Metalloproteinase -2 and -9 in Traumatic Brain Injury

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Abstract : Traumatic brain injury (TBI) is one of the most significant cause for mortality and morbidity in young population. After the primary injury, there is secondary injury that will aggravate the injury and lead to cell death. Secondary injury can be prevented and reversible, providing opportunity for therapeutic intervention. Matrix metalloproteinases (MMPs) are endopeptidases that participates mainly in the dynamic modulation of the extracellular membrane. In some neurological diseases, MMPs will cause breakage of the blood brain barrier (BBB), hemorrhage, neuronal inflammation, and neuronal cell death. In this study, we used an experimental mouse model of TBI to examine the role of MMP-2 and MMP-9 and the therapeutic potential of mangosteen extract that contain natural MMP inhibitor. We observed that expression of MMP-2 and MMP-9 were reduced in treatment group. These findings suggested that mangosteen extract might be a potential therapeutic agent for TBI.
Keywords : Mangosteen extract; MMP-2; MMP-9.

Introduction

Traumatic brain injury (TBI) accounts for significant mortality and morbidity, especially in young population. The overall incidence rate of this condition was 262 per 100,000 populations with case fatality rate ranged from 29 to 55 per 100 in severe cases[1]. Almost all survivor of severe TBI never have full social independence, even without physical disabilities. It was estimated that around 3.17 million US civilian population lived with long term disability after TBI[2]. Jacobs reported that more than half of the survivors were dependent to their family, neither worked nor attended school, implying great burden on families who take care the injured family. Mood disorders are common in this period and there is increase in risk of suicide. In follow up between 2 and 7 years post injury, there is little improvement of this psychological problems, bring lifetime burden on the families[3].

TBI itself is a complicated process, not just a single pathophysiological event[4]. There are two mechanisms occurred in this process, the primary injury and secondary injury. Primary injury is resulted from the mechanical disruption of brain tissue and includes contusion, hemorrhage, and axonal shearing. After the primary injury, there are cascade of metabolic, cellular, and molecular events that lead to brain cell death[5].

Matrix metalloproteinases (MMPs) are endopeptidases that participates mainly in the dynamic modulation of the extracellular membrane[6]. In normal condition, they play crucial role in normal growth, development, wound healing, angiogenesis, and neurogenesis. However, in some neurological diseases, MMPs will cause breakage of the blood brain barrier (BBB), hemorrhage, neuronal inflammation, and neuronal cell death[7].

Studies have indicated the upregulation of MMPs following TBI, mostly MMP-2 and MMP-9. MMP-2 and 9 were increased in cortical astrocyte cell culture after mechanical scratch injury[8]. In human, after severe head injury, MMP-9 was also increased in contusional and pericontusional brain[9] as well as in cerebrospinal fluid (CSF) [10]. Furthermore, some studies have proposed that TBI activates MMPs and may play important role in degradation of ECM, breakage of the BBB, leukocyte infiltration, hemorrhage, cerebral edema, neural inflammation, and degeneration of the neural cell[11]. Regulation of MMP is then considered as one of possible target for initiating therapy to halt secondary brain injury. MMP-9 knock-out mice had less motor deficits than wild-type mice after controlled cortical impact[12]. Selective inhibitor of MMP-9 mitigated microglial activation and astrogliosis after TBI. Importantly, this inhibitor treatment improved long-term neurobehavioral outcomes, including sensorimotor function, and hippocampus-associated spatial learning and memory[13].

Mangosteen, Garciniamangostana Linn, is kind of fruits that can easily be found in South and Southeast Asia. The fruit is dark purple or reddish in color and contains edible white pulps inside that are soft and juicy[14]. The pericarp of this fruit has been used for centuries in South and Southeast Asia for the treatment of skin infection, wounds, diarrhea, and cholera[15]. It has been proven to have anti-inflammatory effect[16]. Alpha mangostin, the xanthone isolated from the pericarp, has been proven to suppress MMP-2 and MMP-9 in head and neck squamous cell carcinoma[17] as well as in pancreatic tumor[18]. However, to this date, no data is available about the effect of mangosteen extract on the MMP-2 and MMP-9 expression in traumatic brain injury. The purpose of this study is to investigate the effect of mangosteen extract on MMP-2 and MMP-9 expression in brain after traumatic brain injury

Material and Method

Mangosteen extract (ME) preparation

Mangosteen were collected from Malang regency in East Java, Indonesia. The fruit bodies of mangosteen were cleaned to remove any residual compost. The pericarp were separated and then dried. All dried pericarp were placed in 70% ethanol (100 gr in 500 cc), shake with speed 50 rpm in 24 hours. The macerate filtrate was evaporated using rotary evaporator in 2 hours. From 100 gr dried mangosteen pericarp, 50 cc ME was obtained.

Apparatus and application of the method of TBI

Thirty Sprague dawley rats weighing 250-400 gr were used in these experiments. We used the modified Feeney's weight-drop model for making TBI[19]. All animals were given ketamine HCl (100 mg/kg, intramuscular) as anesthetic agent. The scalp was cleaned with Povidone Iodine and aseptic techniques were used throughout surgery. The scalp was opened on the right frontal. Then, the rats were placed securely in stereotactic apparatus. We gave 40 mg metal mass from 1.5 m height. For the treatment group, we gave ME (100 mg/kg per oral) for five days. Afterward, animals were sacrificed through cervical dislocation after giving ketamine HCl (100 mg/kg, intramuscular). The brains were removed and fixed in 10% formalin. The specimens were then processed for paraffin-embedded for immunohistochemistry staining.

Immunohistochemistry staining

The expression of MMP-2 and MMP-9 were investigated on paraffin-embedded sections using the avidin-biotin-peroxidase complex method. Five-millimeter-thick paraffin sections were dewaxed, rehydrated, and microwave for 10 minutes. The endogenous peroxidase activity of the investigated specimens was blocked with 3 percent H2O2 for 10 minutes, followed by 25-minute washing with phosphate-buffered saline (PBS). The tissue sections were incubated with normal rabbit serum for 10 minutes, and then the slides were incubated at room temperature with monoclonal mouse anti-human MMP-2 and MMP-9 (Santa Cruz). Sections were washed with PBS and incubated with a secondary antibody for 30 minutes. Sections were washed twice with

PBS, developed with 0.05 percent 3,3diamino-benzinetetrahydrochloride for five minutes, and slightly counterstained.

All samples were evaluated by one pathologist (blinded) and first author (not blinded to specimen). Positive signal for MMP-2 was located in the cytoplasm of brain cells and the stainability was semiquantitatively estimated on the basis of distribution of positive stained tumor cells and the staining intensity(20). Based on the distribution of positive stained tumor cell, samples were divided into four groups, i.e.0 (if there was no stained cell), 1 (if less then 25% cells were stained), 2 (if 25-49% cells were stained), 3 (if 50-75% cells were stained), and 4 (if more than 75% cells were stained). Based on the staining intensity, samples weredivided into three groups, i.e. 1 (if the intensity was weak), 2 (if the intensity was moderate), and 3 (if the intensity was strong).

Statistical Analysis

Distribution and intensity were reported in percentage. When comparisons were made between groups, significance in between-group variability was analyzed using the Kruskal-Wallis test. For post-hoc test, The Mann- Whitney *U* test was used to analyze the difference between groups. Differences were considered significant at the $P < 0.05$

Result

Thirty rats were included in this research, divided into three groups, i.e. negative control group (without trauma, without ME), positive control group (with trauma, without ME), and treatment group (with trauma, with ME). ME were given consecutively in five days. During the follow up, two rats died directly after trauma procedure. The brain was removed after craniocervical dislocation (figure 1).

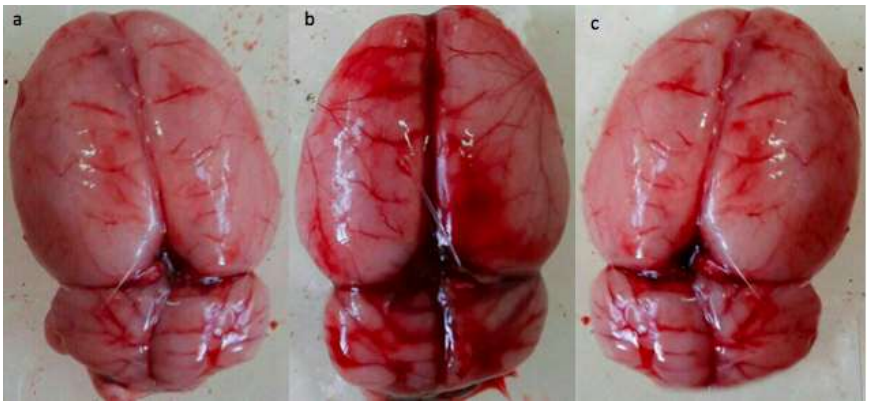

Figure 1.Macroscopic brain of the rats. (a). Negative control group; (b). Positive control group; (c) Treatment group

MMP-2 expression

MMP-2 immunoreactivity presented as diffuse cytoplasmic staining in brain cells (figure 2). In the negative control group, distribution of positively stained cells were +2 in 11,1% and +3 in the rest. In positive control group, distribution of positively stained cells were +3 in 22,2% and +4 in 77,8%. In the treatment group, distribution of positively stained cells were +3 in 77,8% and +4 in 22,2%. There was significant difference between distribution of MMP-2 positive cells among all groups (p=0.002).

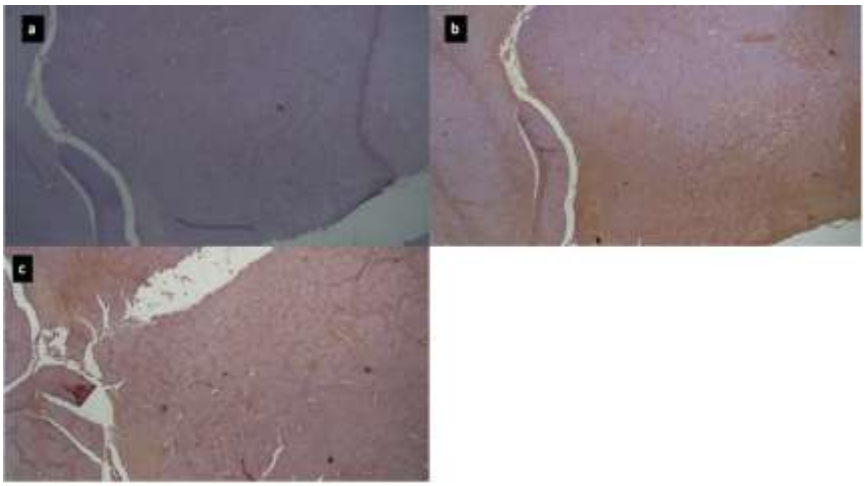

Fig. 2.MMP-2 expression in (a).Negative control group, (b).Positive control group, (c). Treatment group

After post hoc test, there were significant difference between group 1 and group 2 (p=0,003) as well as between group 2 and 3 (p=0.050). There was no significant difference between group 1 and 3 (p=0.297, table 1).

There was no significant difference among the three groups regarding staining intensity (p=0.538, table 1).

Table 1. MMP 2 expression (n=9)

	Group 1 (n,%)	**Group 2 (n,%)**	**Group 3 (n,%)**	**p**
Distribution				
0	-	-	-	0.002*
+1	-	-	-	
+2	1 (11.1)	-	-	
+3	8 (88.9)	2 (22,2)	7 (77,8)	
+4	-	7 (77,8)	2 (22,2)	
Intensity				
+1	-			0.538
+2	8 (88,9)	7 (77,8)	6 (66,7)	
+3	1 (11,1)	2 (22,2)	3 (33,3)	

MMP-9 expression

MMP-9 immunoreactivity presented as diffuse cytoplasmic staining in brain cells (figure 2). In the negative control group, distribution of positively stained cells were +1 in 11.1% and +2 in the rest. In positive control group, distribution of positively stained cells were +2 in 11.1% and +3 in 66.7%, and +4 in 22.2%. In the treatment group, distribution of positively stained cells were +1 in 33.3%, +2 in 55.6%, and +3 in 11.1%. There was significant difference between distribution of MMP-2 positive cells among all groups (p=0.0001).

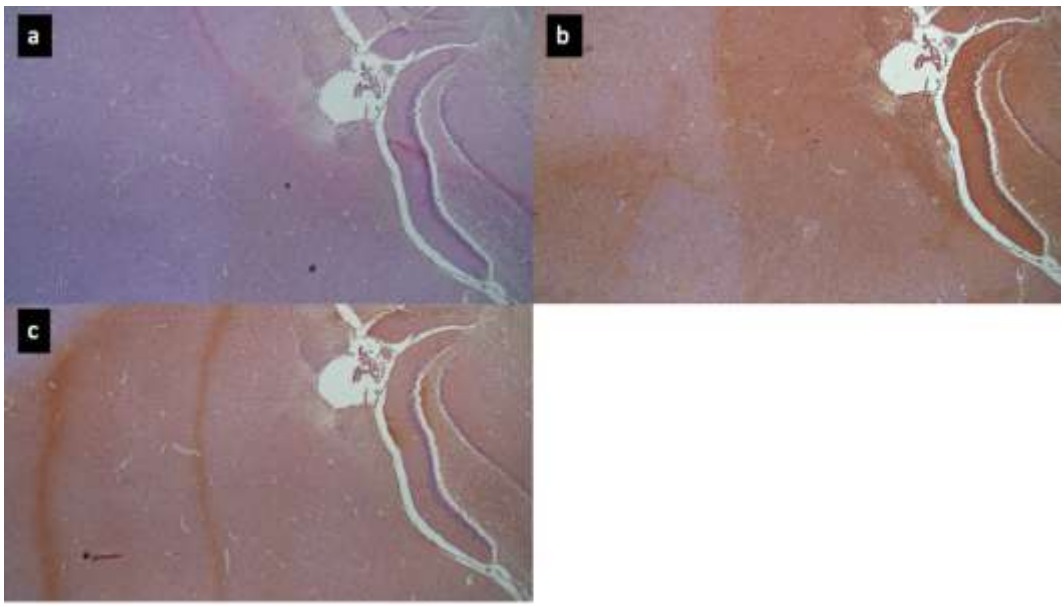

Fig. 3. MMP-9 expression in (a). Negative control group, (b). Positive control group, (c). Treatment group

After post hoc test, there were significant difference between group 1 and group 2 (p=0,0001) as well as between group 2 and 3 (p=0.001). There was no significant difference between group 1 and 3 (p=0.666, table 2).

Table 2. MMP 9 expression (n=9)

	Group 1 (n,%)	**Group 2 (n,%)**	**Group 3 (n,%)**	**p**
Distribution				
0	-	-	-	0.0001*
+1	1 (11,1)	-	3 (33,3)	
+2	8 (88,9)	1 (11,1)	5 (55,6)	
+3	-	6 (66,7)	1 (11,1)	
+4	-	2 (22,2)	-	
Intensity				
+1	9 (100)	3 (33,3)	7 (77,8)	0.008*
+2	-	6 (66,7)	2 (22,2)	
+3	-	-	-	

There was significant difference among the three groups regarding staining intensity (p=0.008, table 2). After post hoc test, there were significant difference between group 1 and group 2 (p=0,014). There was no significant difference between group 1 and 3 (p=0.436) as well as between group 2 and 3 (p=0.113, table 2).

Discussion

TBI is associated with a wide scope of symptoms and disabilities. Beside the initial primary brain injury, there is also secondary brain injury that happen after the initial injury. Unlike the primary brain injury, it is a complex process that theoretically could be prevented and may be reversible. In secondary brain injury, there is a series of molecular, neurochemical, and cellular mechanism that may conduct to elevated intracranial pressure, BBB breakage, inflammation of the neural cell, cerebral edema, brain hypoxia, ischemia, and delayed neurodegeneration[21-23]. The MMPs represent one of the most prominent family associated with secondary brain injury. Recent knowledge has advanced our understanding about the role of MMPs as modulators of the traumatic brain injury. Abnormal MMP activation post trauma degrades the microvascular basement membrane

proteins, resulting the breakage of blood brain barrier and cerebral edema[24]. Further studies suggested that MMP-2, MMP-3, and MMP-9 are capable to activate the interleukin 1b[25]. MMPs also stimulates production of vascular endothelial growth factor (VEGF). Increased VEGF will activate caspase-1 and cause apoptosis[26]. This huge range of MMPs' effect in TBI makes it become one of ideal targets in managing TBI.

A number of synthetic inhibitors have been developed. Most of them was made by adding zinc-binding globulin (ZBG). ZBG will displace zinc-bound water molecule and inactivate MMPs[27]. Besides that, there are several natural MMP inhibitors derived from various resources such as herbs, plants, fruits, and other agriculture products. These natural MMP inhibitors include long chain fatty acids, epigallocatechingallate (EGCG) and other polyphenols, flavonoids, and a variety of other natural compounds[28]. Mangosteen was proven to suppress MMP-2 and MMP -9 in squamous cell carcinoma[17]. α-mangostin, the active component of ME, was distributed to brain after given orally[29]. It was proven to reduce oxidative damage in brain tissue[30] and accelerate amyloid-beta clearance in Alzheimer's disease[31]. To explore the effect of ME in MMP-2 and MMP-9 expression, we conducted this experiment. The result showed that there was significant difference in MMP-2 and MMP-9 expression between positive control group and treatment group, especially in MMP-9. MMP-2 and MMP-9 belong to gelatinases. Both are found in the extracellular matrix, cerebrospinal fluid, and serum[8-10]. Upregulation of MMP-2 seemed to be happened in shorter duration compared to MMP-9. Abdul-Muneer et al (2013) reported that MMP-2 level would significantly decrease after 6 hours, unlike MMP-9 that persistently increased in first 24 hours[32].

The expression of MMP-2 and MMP-9 gene is under the control of several growth factor and cytokines. Several studies have revealed the involvement of various signaling pathways in MMP-2 and MMP-9 regulation depending on the cell types and nature of the stimuli. The signaling pathways via MAPKs, NF-κB, and PI3K/Akt have been reported in cancer[33]. In traumatic brain injury, secretion of MMP-9 was mediated by the upregulation of ERK and p38 MAP kinase[8]. Therefore, investigations of signaling pathways and molecular mechanism associated with the effect of ME on MMP-2 and MMP-9 expression in TBI should be performed and required for further study.

The main limitation of this study is the nature of the TBI process. Using the weight drop models, we could only make focal injury[34]. Diffuse injury itself could also contribute to acute cognitive, motor, and affective disturbance[35]. We also only gave the ME in crude form, not the active xanthone. Mangosteen has several xanthones contained in the fruit, pericarp, trunk, branches, and leaves[15].

In conclusion, to the best of our knowledge, this study is the first report to demonstrate that ME decreases MMP-2 and MMP-9 expression in traumatic brain injury. From these results, it was suggested that mangosteen might be a potential therapeutic agent in traumatic brain injury. However, further in vitro study should be performed.

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Conflict of Interest

Archives of Physical Medicine and Rehabilitation

The authors declare that they have no conflict of interest.

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