



## **The Effect of Curcumin on Cognitive Dysfunction Through Cholinergic Pathway in Hippocampus, Frontal Cortex and Caspase 3 in Rat Shohami's Weight-Drop Model Traumatic Brain Injury**

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**Abstract :** TBI (traumatic brain injury) results in significant disability due to cognitive deficits mainly in attention, learning and memory and higher-order executive functions. In this study we examined the effect of Curcumin on cognitive dysfunction through increase ACH and decrease caspase 3 expression in hippocampus and cortex. Twenty five anaesthetized Wistar rats were subjected to TBI using Shohami weight-drop model. The ACH and caspase 3 activity was determined using immunohistochemistry in brain tissue slices after 5 days treatment of Curcumin at 3 different doses 50 mg/kg, 100 mg/kg, 200 mg/kg and examined cognitive function using MWM. Increase expression of ACH was observed in hippocampus and cortex of brain after administration of curcumin in TBI, also was observed decrease expression of caspase 3 and improvement of cognitive dysfunction. In addition to observation above we found that increase expression of ACH no significant difference between treatment group, while decrease expression of caspase 3 was significant with high dose, and leading to good outcome of cognitive dysfunction. From our result we suggest that after TBI administration of curcumin may improve cognitive dysfunction through mechanism involving increasing of expression of ACH and decreasing of expression of caspase 3 and therefore decreasing apoptosis.

**Key words :** Caspase 3, cholinergic, curcumin, TBI.

### **Introduction**

Traumatic Brain Injury can causing brain damage include structural and/or physiological disruption of brain function<sup>1</sup>. One of the most important cognitive sequelae of traumatic brain injury (TBI) is memory impairment<sup>2</sup>. Memory dysfunction after TBI implicated the hippocampus as an important structure for memory function<sup>3</sup>.

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Primary and secondary injuries that occurred in the brain are responsible for cognitive dysfunction following TBI. Many biochemical derangements that are responsible for secondary injury have been identified. These include glutamate excitotoxicity, disorder of cellular calcium homeostasis, increased free radical generation and lipid peroxidation, mitochondrial dysfunction, inflammation, apoptosis and diffuse axonal injury<sup>4,5</sup>.

Activation of caspase-3 induced by two caspase initiator pathways: a caspase-8-mediated pathway activated through cell surface death receptors (extrinsic pathway). And a caspase-9-mediated pathway activated by signals from the mitochondria that lead to formation of an apoptosomal complex<sup>6</sup>. TBI lead to reduction in cholinergic function, which reflected by decreasing of ACH in hippocampus. The cholinergic pathway responsible for process of cognition arousal and attention, therefore any change in this pathway may result in cognitive deficit after TBI<sup>7</sup>. Curcumin is the active ingredient of the dietary spice turmeric and used for medicinal purposes for thousands of years. Curcumin has been shown to have anti-inflammatory, anti-oxidant, pro-apoptotic, chemotherapeutic, anti-proliferative, wound healing, anti-nociceptive<sup>8</sup>.

The aim of this study to observe its effect of curcumin on improving cognitive function (memory) by inhibition caspase 3 and increasing level of ACH in traumatic brain injury, and the potency of curcumin to protect brain from damage.

## Experimental

This study is true experimental design, in this study 25 male Wistar rat where divided into 5 group consisting of 5 rat, including control negative (C-), control positive (C+), T1, T2, T3. Where control negative group without trauma and treatment, control positive group with TBI only, T1 with traumatic brain injury, which was administered intraperitoneal 50 mg /kg body weight curcumin, T2 with TBI which was administered intraperitoneal 100 mg/kg body weight curcumin, T3 with TBI which was administered intraperitoneal 200 mg/kg body weight curcumin.

### Experimental Traumatic Brain Injury Model

Shohami's weight drop model used, the rats were anesthetized, midline incision over the skull performed, the skin retracted, and the skull exposed to locate the area of impact, then 3 mm diameter plastic tip of a metal rod weighting 35 g gently drop from 100 cm height onto exposed skull at the left hemisphere 2 mm lateral from the midline and 2 mm back from the coronal suture<sup>9,10</sup>. To evaluation of neurological motor function deficit in rat after TBI was used Neurological Severity Score (NSS) (Table 1)<sup>11</sup>.

### Cognitive Testing

The test was performed in Morris Water Maze (MWM), round pool contain water with 26<sup>0</sup>C and place platform in center of the pool. The rat trained in the water maze with 12 consecutive trials per day for 3 days. First, put the rat on the platform for twenty seconds. The water maze has 4 starting positions: north, south, east, or west. Take the animal to one of these positions, and rat swim and search for the platform for a maximum of 60 seconds, when mice reaches the platform, stop the timer, and record the time<sup>11</sup>.

### Immunohistochemistry

After finished of treatment, the rats sacrificed, the brain tissue removed and put in formalin 10% not more than 24 hours, the following steps are dehydration, clearing, embedding, block in paraffin. The tissue was cut with thickness of 6  $\mu$ m using microtome. Immunohistochemistry staining was done to see caspase 3 and ACH distribution in hippocampus and cortex. At first slide incubate at 60C for 1 hour, wash specimen with dH<sub>2</sub>O 3 time for 5 minute apply 3% H<sub>2</sub>O<sub>2</sub> in methanol for 15 minute at room temperature, then wash specimen 3 time of PBS for 5 minute. Dilution (1:1000) primer antibody anti caspase 3 in blocking buffer, then incubate overnight at 40 C. Wash specimen 3 time of PBS for 5 minute. Apply secondary antibody to specimen for hour at room temperature, wash specimen for 3 time of PBS for 5 minute. Apply SA-HRP to specimen for 45 minute at room temperature, then wash 3 time of PBS for 5 minute, and wash once in H<sub>2</sub>O for 1 minute. Dilute DAB chromagen in DAB buffer 1:50 pipet into section 20 $\mu$ l/section, stain for 10-20 minute at room temperature. Wash specimen 3 time of dH<sub>2</sub>O for 5 minute. Apply Mayer haematoxylin in tap water to specimen for 5 minute,

wait until specimen to dry. Immunohistochemistry staining were examined by Olympus microscope, by observing brown colour seen in 20 field of views with magnification of 1000x.

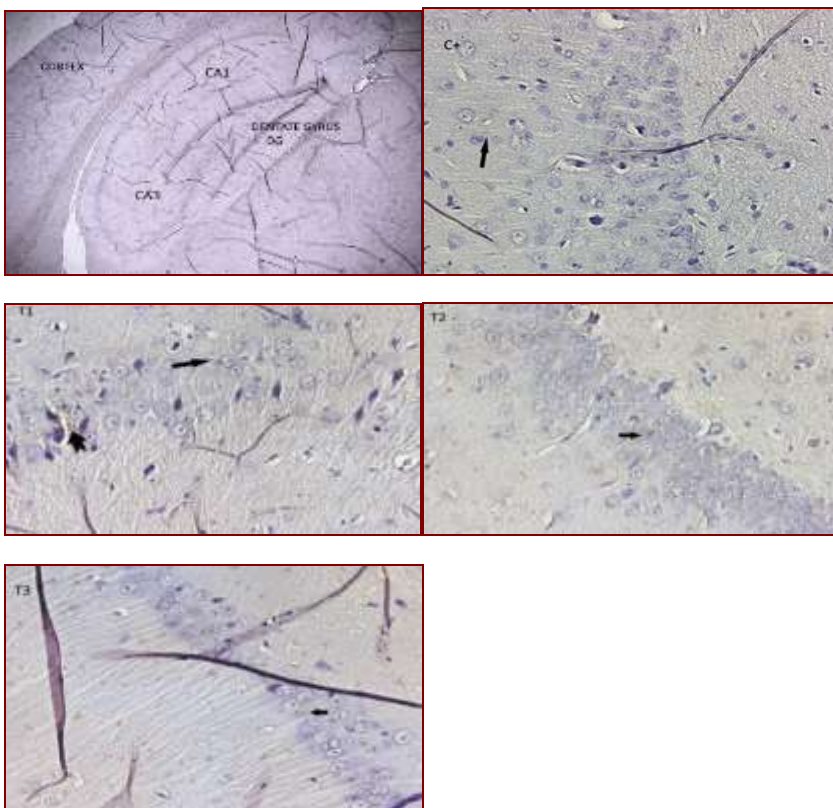
### Statistical Analysis

All data was subjected to statistical analysis. We analyzed our data using One-Way ANOVA test followed by Post-Hoc test (LSD), a significant  $p < 0.05$ , to assess the comparison test followed by Post-Hoc test (LSD), a significant  $p < 0.05$ , to assess the comparison between groups. We considered  $< 0.05$  as significant.

## Results

### Expression of ACH in Hippocampus

As shown in Figure 1 that control positive group expression of ACH in hippocampus after TBI decreased compared with treatment group, but expression of ACH between treatment group was more expressed in T3. Positive expression noted with brown color in hippocampus.



**Figure 1. The effects of TBI in the distribution of ACH on hippocampus. Immunohistochemistry was done by DAB staining (brown color). Compared to the control group (C+,-), ACH expression decrease after TBI (C+), while ACH expression in treatment groups increased (T1, 2, 3). Arrows indicate expression of ACH.**

Based on Figure 2 below shows that giving curcumin at 100 mg/kg (T2) can increase the average of expression of ACH in hippocampus rather than the provision of curcumin at a dose at 200 mg/kg (T3), so dose 100 mg/kg (T2) was more effective to increase expression of ACH in hippocampus. However, the provision of curcumin at 200 mg/kg (T3) can increase the average expression of ACH in hippocampus better than dose of 50 mg/kg (T1), so T3 was more effective to increase expression of ACH in hippocampus than T1. But the average of expression of ACH in hippocampus TBI in granting curcumin at T1 was higher than the positive control group, so this group is effective to increase expression of ACH in hippocampus.

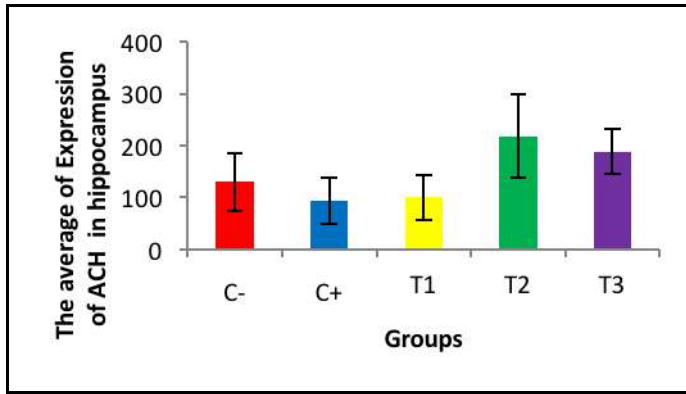


Figure 2. Expression of ACH in hippocampus at each dose of curcumin. (C-) control negative, (C+) control positive, T1 (50 mg/kg curcumin), T2 (100 mg/kg), T3 (200 mg/kg)

### Expression of ACH in Cortex

In Figure 3 shown that in control positive group animal no observable expression of ACH in the cortex of brain, but in other group treatment of animal was observable expression with different degree, as we observed in group T3 at dose of curcumin 200 mg/kg more expression compared with others.

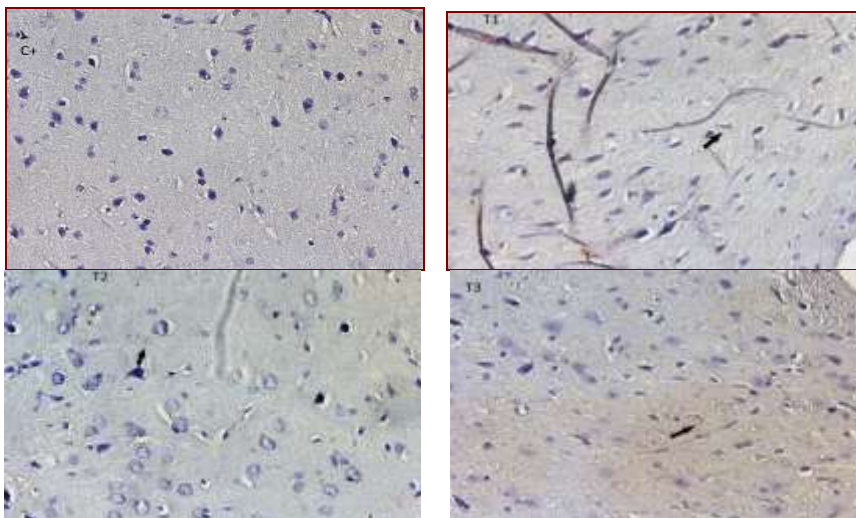


Figure 3. The effects of TBI in distribution of ACH in the cortex of brain tissue. The figure shows the immunohistochemistry staining visualized by DAB (brown). Compared to the control group (C+,-), ACH expression decrease after TBI (C+), while ACH expression in treatment groups increased (T1, 2, 3). Arrow indicate expression ACH in cortex.

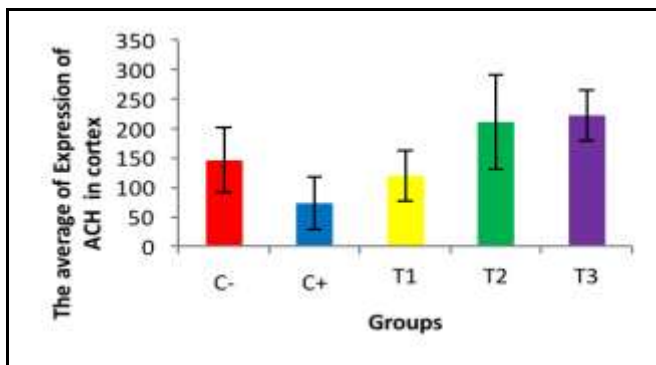


Figure 4. Expression of ACH in cortex at each dose curcumin (C-) control negative, (C+) control positive, T1 (50 mg/kg curcumin), T2 (100 mg/kg),T3 (200 mg/kg)

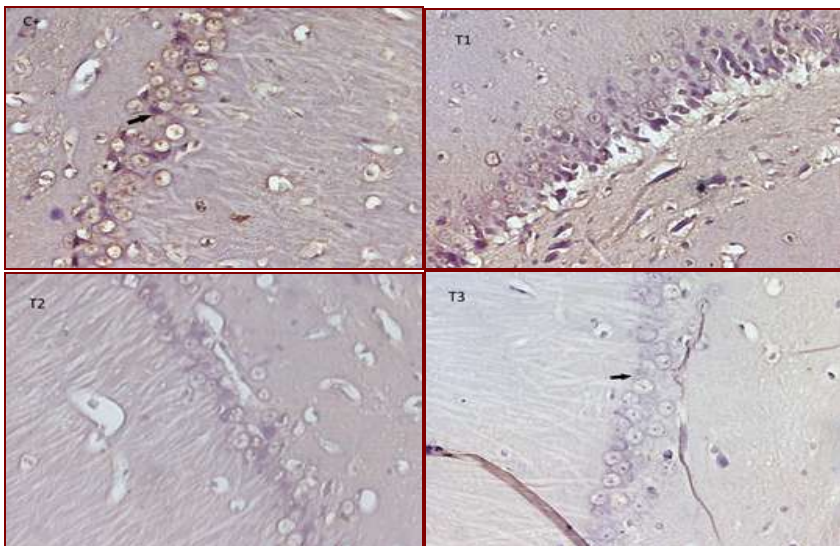


Based on Figure 4 below shows that giving curcumin at dose 200 mg (T3) can increase the average of expression of ACH in cortex rather than the provision of curcumin at a dose at dose Of 100 mg (T2), so T3 was more effective to increase expression of ACH in cortex than T2. However, the provision of curcumin at T2 can increase the average expression of ACH in cortex better than dose 50 mg (T1), so T2 was more effective to increase Expression of ACH in cortex than T1. But the average of expression of ACH in cortex TBI in granting curcumin at T1 was higher than the positive control group, so this group is effective to increase expression of ACH in cortex.

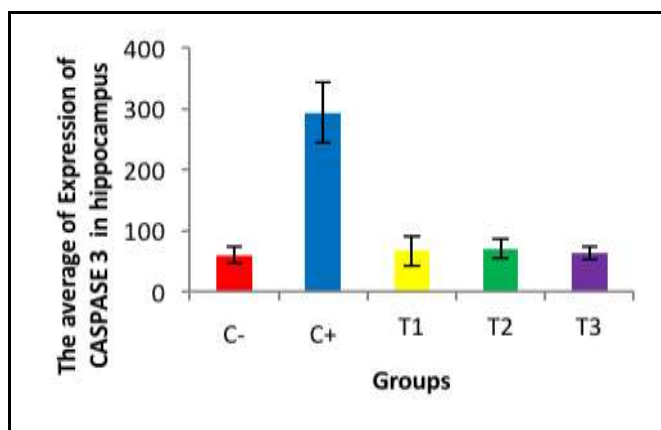
From data above after we administered different concentrations of curcumin (50, 100, or 200 mg/kg) to rats post-TBI for 5 days, and examine ACH expression in hippocampus and cortex. The administration of 3 doses curcumin did not significantly increase expression of ACH in hippocampus compared with TBI alone ( $P > 0.05$ ). In contrast, increase expression of ACH in the cortex of brain at 100 mg/kg or 200 mg/kg curcumin significantly increase ACH expression ( $P < 0.05$ ).

**Expression of Caspase 3 in Hippocampus:**

As shown in Figure 5 in control positive group that expression of caspase 3 in hippocampus after TBI, was observable and more intense compared with other group, where in group treatment T1, T2, T3 also observed that more dose of curcumin, less intense of expression of caspase 3 in hippocampus.



**Figure 5. The effects of TBI in distribution of caspase 3 in Hippocampus. The figure shows the immunohistochemistry staining of visualized by DAB (brown). Compared to the control group (C+,-), Caspase3 expression increase after TBI (C+), while caspase 3 expression in treatment groups increased (T1, 2, 3).**



**Figure 6. Expression of caspase 3 in hippocampus at each dose curcumin (C-) control negative, (C+) control positive, T1 (50 mg/kg curcumin), T2 (100 mg/kg), T3 (200 mg/kg)**

Based on Figure 6 above shows that giving curcumin at 200 mg (T3) can decrease the average of expression of caspase 3 in hippocampus rather than the provision of curcumin at a lower dose at 50 mg (T1) and 100 mg T2, so T3 was more effective to decrease expression of caspase 3 in hippocampus than T1 and T2. However, the provision of curcumin at T2 can decrease the average expression of caspase 3 in hippocampus better than 50 mg (T1), so T2 was more effective to decrease expression of caspase 3 in hippocampus than T1.

**Expression of Caspase 3 in Cortex**

As shown in Figure 7 in control positive group that expression of caspase 3 in cortex of brain after TBI and was observable and more intense compared with other group, where in group treatment T1, T2, T3 also observed that more dose of curcumin, less intense of expression of caspase 3 in hippocampus thus in group T3 the expression of caspase 3 less intense compared with group T1 and T2.

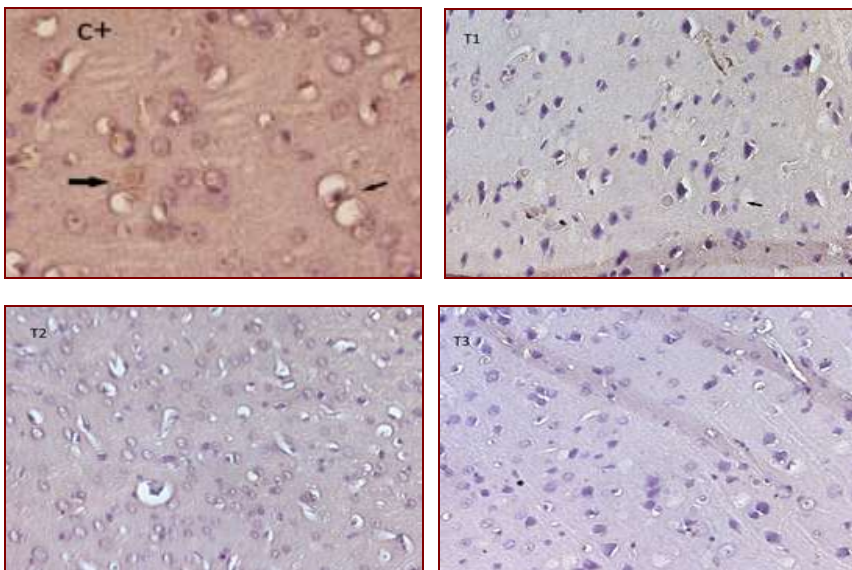


Figure 7. The effects of TBI in distribution of caspase 3 in cortex. The figure shows the immunohistochemistry staining of visualized by DAB (brown). Compared to the control group (C+,-), caspase 3 expression increase after TBI (C+), while caspase 3 expression in treatment groups increased (T1, 2, 3).

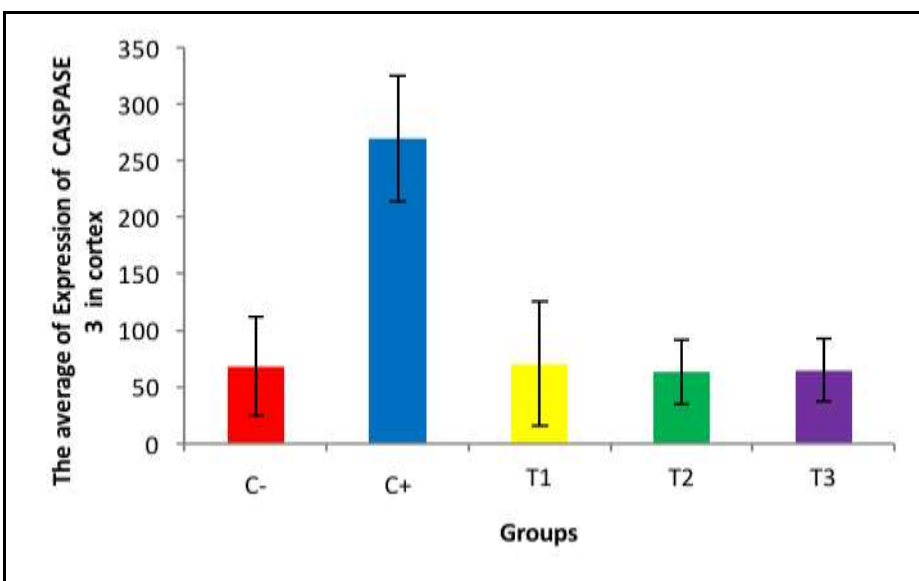


Figure 8. Expression of caspase 3 in cortex at each dose of curcumin. (C-) control negative, (C+) control positive, T1 (50 mg/kg curcumin), T2 (100 mg/kg), T3 (200 mg/kg)

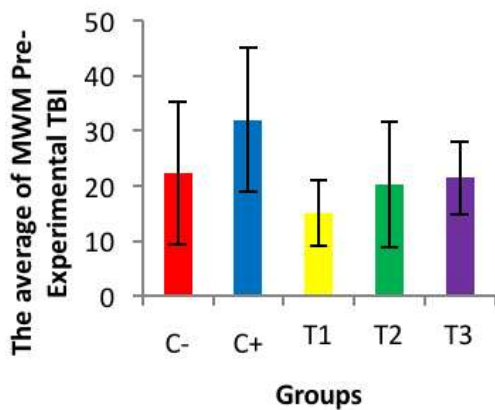
Based on above giving curcumin at dose of 200 mg (T3) can decrease the average of expression of caspase 3 in cortex rather than the provision of curcumin at a dose at 100 mg (T2), so T3 was more effective to decrease expression of caspase 3 in cortex than T2. However, the provision of curcumin at T2 can decrease the average Expression of caspase 3 in cortex better than 50 mg (T1), so T2 was more effective to decrease expression of caspase 3 in cortex than T1.

From data above after we administered different concentrations of curcumin (50, 100, 200 mg/kg) to rats post-TBI for 5 days, and examine caspase 3 expression in hippocampus and cortex. The administration of 3 doses curcumin was significantly decrease expression of caspase 3 in hippocampus compared with TBI alone ( $P > 0.05$ ) also decrease expression of caspase in the cortex of brain at 3 different doses of curcumin was significantly decrease caspase 3 expression ( $P < 0.05$ ).

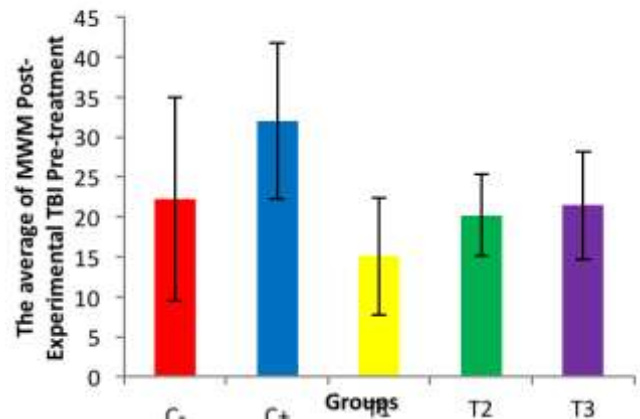
**MWM Pre-Experimental and Post-Experimental TBI Post-treatment**

From data on Figure 9 shows that giving curcumin at 200 mg (T3) can decrease of MWM Post-Experimental TBI Post-treatment rather than the provision of curcumin at a lower dose at 100 mg (T2), so dose T3 was more effective to decrease MWM Post-Experimental TBI Post-treatment than T2. However, the provision of curcumin at 100 mg (T2) can decrease the average MWM Post-Experimental TBI Post-treatment better than 50 mg (T1), so dose T2 was more effective to decrease MWM Post-Experimental TBI Post-treatment than dose T1.

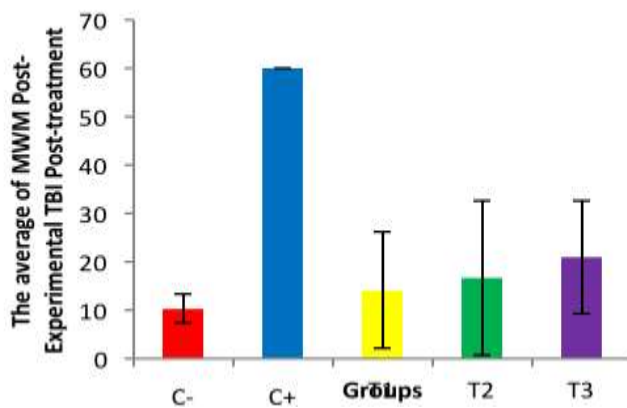
A.



B.



C.



**Figure 9. MWM Post-experimental TBI Post-treatment at each dose curcumin**

## Discussions

### Effect of Curcumin on ACH and Cognitive Dysfunction in TBI

In this study, we used curcumin to observe its effect in rats post TBI to improve cognitive dysfunction through increase ACH and decrease caspase 3 which implicated in this dysfunction, TBI can result in cognitive deficits mainly in attention, learning and memory<sup>4</sup>. Outcome of TBI determine by different stage of injury: primary and secondary damage, where primary damage occur at moment of trauma, while secondary damage is consecutive processes that begin at moment of injury<sup>12</sup>. After TBI can occur reduction in cholinergic function by decreasing of ACH in hippocampus that responsible for cognition<sup>13</sup>.

In this study used curcumin in 3 various dose, to investigate about the effect of curcumin on ACH, caspase 3 and cognitive dysfunction after TBI. In our study after administration curcumin in various doses, the result showed that curcumin can increase expression of ACH in hippocampus. Giving curcumin at dose of 100 mg/kg can increase expression of ACH in hippocampus rather than the provision of curcumin at a dose at 200 mg/kg, so dose of 100 mg/kg was effective to increase expression of ACH in hippocampus than 200 mg/kg. However, the provision of curcumin at 200 mg/kg can increase expression of ACH in hippocampus better than 50 mg/kg. While in cortex giving curcumin at dose of 200 mg/kg can increase of expression of ACH in cortex rather than the provision of curcumin at a dose at 100 mg/kg, thus the dose of 200 mg/kg was more effective to increase expression of ACH in cortex than 100 mg/kg. However, the provision of curcumin at 100 mg/kg can increase Expression of ACH in cortex better than dose of 50 mg/kg, thus dose 100 mg/kg was more effective to increase expression of ACH in cortex than 50 mg/kg. As mention before ACH play important role in cognitive function, but in TBI can causing cholinergic dysfunction leading to deficits in learning and memory due to cholinergic neuronal loss in many area of brain<sup>14</sup>. The mechanism how cholinergic neuron susceptible to trauma unknown, but it is suggested that disruption in nerve growth factor production and transport to these neurons, another suggestion that these neurons susceptible to excitotoxic of potassium and glutamate which release after TBI<sup>15</sup>. From this point it is suggested that curcumin play role in prevent or neuroprotection in TBI. There was study<sup>16</sup> about dietary curcumin in TBI on oxidative stress, synaptic plasticity, and cognition, and this study reported that curcumin has antioxidant effect and can reduce oxidative damage on synaptic plasticity and cognitive function.

### Effect of Curcumin on Caspase 3 and Cognitive Dysfunction in TBI

Curcumin at dose 200 mg/kg can decrease expression of caspase 3 in hippocampus rather than the provision of Curcumin at a lower dose at dose 50 mg/kg and 100 mg/kg, thus dose 200 mg/kg was more effective to decrease expression of CASPASE 3 in hippocampus. curcumin at 3 different doses 50 mg/kg, 100 mg/kg and 200 mg/kg can decrease expression of caspase 3 in cortex. In this study we found decreasing expression of caspase 3 in hippocampus more at higher dose, while decreasing expression of caspase 3 in cortex with different doses, we found effect is the same. Where apoptosis increase due to oxidative stress that induce production free radical, hydrogen peroxide, nitric oxide in TBI. Excessive production of reactive oxygen species due to deficit of endogenous antioxidant system, causing immediate cells death and early or late apoptosis<sup>12</sup>, and this data suggest that curcumin act through its antioxidant effect.

### Correlation between ACH, Caspase 3 and Cognitive Dysfunction

At first there is correlation between doses of curcumin and expression of ACH and expression of caspase 3 in cortex and hippocampus. The expression of ACH in hippocampus and cortex have coefficient of correlation equals to 0.503 and 0,690 respectively, which shows us relation quite strong. Also correlation between doses of curcumin and expression of caspase 3 in hippocampus and cortex and have coefficient of correlation equals to -0,669 and -0,636 respectively, and it's quite strong. That mean the increase of doses of curcumin will be followed increase of expression of ACH in hippocampus and cortex, and will be decrease of expression of caspase 3 in hippocampus and cortex, and therefore will improve cognitive function.



## Conclusion

Our finding suggest that curcumin administration may improve cognitive dysfunction outcome through mechanism increase ACH, and decrease activity of caspase 3 (neuronal apoptosis) at dose high as 200 mg/kg of curcumin.

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## Conflict of Interest

Author declare no conflicts of interest

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