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Tualang honey attenuates glutathione depletion in the rat hippocampus following kainic acid administration

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Abstract

Excitotoxicity mediated neurodegeneration by kainic acid (KA) was shown to cause oxidative stress in rats' brains. Tualang honey (TH), a potential natural medicinal agent, was reported to have many therapeutic properties; however, its protection against neurodegenerative disorders was limited. This study aimed to investigate the protective effects of TH on glutathione levels following KA administration in the rats' hippocampus. Sprague Dawley male rats (n=24) were randomly divided into four groups which are: (i) control, (ii) KA alone, (iii) TH + KA, and (iv) Topiramate (TPM) + KA, and each group was pre-treated orally with either distilled water, TH (1.0 g/kg) or Topiramate (40 mg/kg), respectively, five times at 12 h intervals. Saline or KA (15 mg/kg body weight) were injected subcutaneously 30 min after the last oral treatment. All animals were sacrificed 24 h after KA injection and their hippocampus was harvested to assay the level of reduced glutathione (GSH), oxidized glutathione (GSSG), and GSH:GSSG ratio by using commercially available ELISA kits. The result showed a significant ($p<0.05$) decrease in the level of GSH in the KA alone group and was improved by TH pre-treatment. Meanwhile, the elevation of GSSG level in the KA-induced group was significantly ($p<0.05$) reduced by pre-treatments of TH and Topiramate. Remarkably, the pre-treatment of TH was significantly ($p<0.05$) increases the GSH:GSSG ratio after KA administration. In conclusion, TH showed potential protective effects to prevent oxidative stress-related consequences by attenuating the glutathione system in the rats' hippocampus after KA administration.

Keywords: Tualang Honey; Glutathione; Kainic Acid; Rat Hippocampus

Tualang Honey Attenuates the Glutathione Depletion in the Rat Hippocampus Following Kainic Acid Administration

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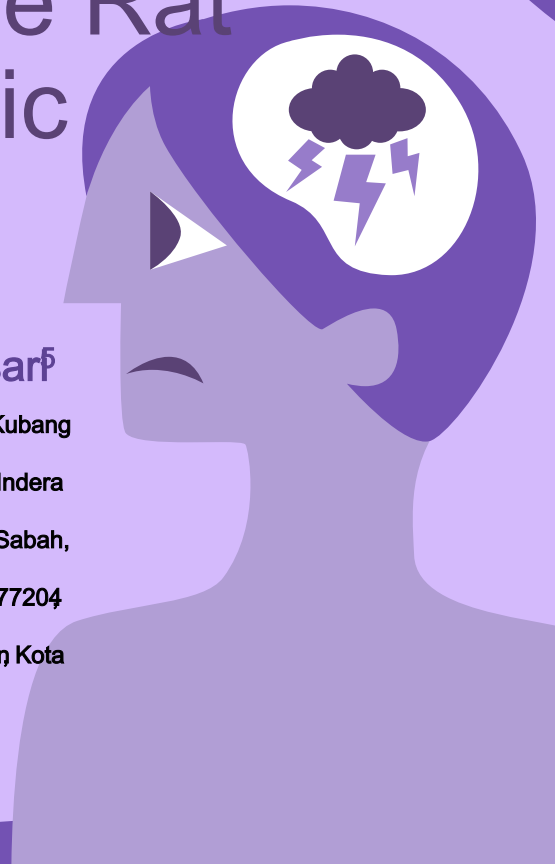

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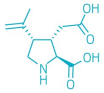
1. Introduction

Tualang honey

- Tualang honey (TH), mostly found in forests of Peninsular Malaysia, collected from the combs of Asian rock bees "Apis dorsata", the world's largest honeybees [1].
- TH is a natural antioxidant agent that contains higher **polyphenols** and **flavonoids** compare to other Malaysian honey (2].
- Studies on TH in human and animal models, indicated that it may have medicinal properties such as **antibacterial** [3], **antioxidant** [4], **anticancer** [5], **antidiabetic** [6] and potential protection against **neurological disorders** [7].
- Due to the potential applicability of TH for therapeutic purposes, numerous studies are focusing on the mechanisms of antioxidant action of TH on excitotoxicity-induced neurodegenerative models

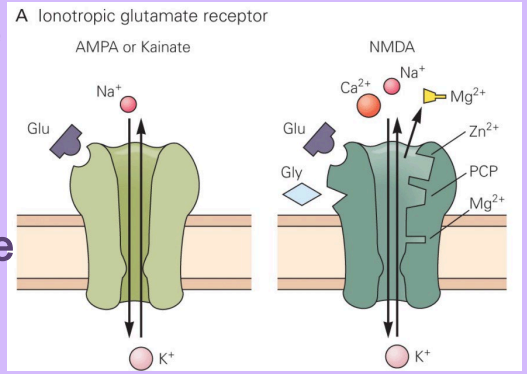
Excitotoxicity

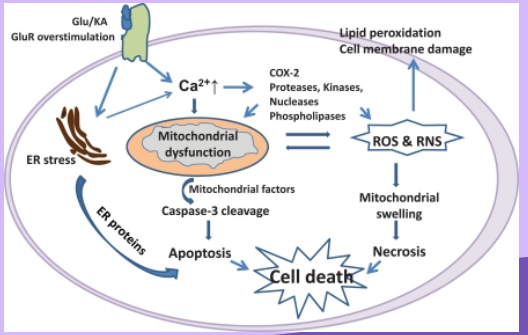
- Excitotoxicity is considered to be an important mechanism involved in various neurodegenerative diseases in central nervous system (CNS), such as, **Alzheimer's disease** [8], **Parkinson's disease** [9] **amyotrophic lateral sclerosis** [10] and **epilepsy** [11].
- Excitotoxicity is a phenomenon that describes the toxic actions of excitatory neurotransmitters, primarily glutamate, where the **prolonged activation of glutamate receptors** starts a cascade of neurotoxicity that ultimately leads to the loss of neuronal function and cell death [12].
- Excitotoxicity is commonly induced experimentally by chemical convulsants, particularly **kainic acid**



Kainic acid

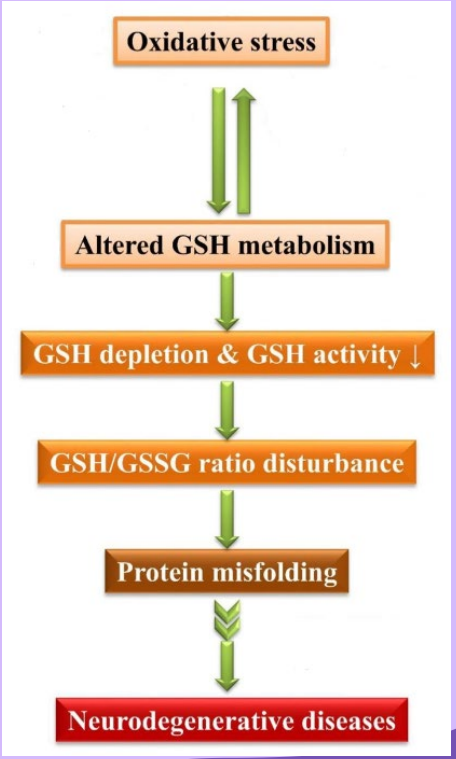
- Kainic acid (KA) is a specific agonist of **ionotropic glutamate receptors** (iGluRs) and a strong **neurotoxin**.
- KA acts on kainate receptors (KARs) in the CNS and **imitates the excitotoxin** action of glutamate in models of neurodegenerative disorders [13].
- The KA binding to KARs causes a number of cellular events, including the **influx of Ca^{2+}** into cells, the **production of reactive oxygen species** (ROS) and reactive nitrogen species (RNS), which leads to the **mitochondrial dysfunction**, **apoptosis** of neurons and **necrosis** [13].
- Activation of KA receptors has been reported to cause **decreases in the glutathione (GSH)** pool in a number of brain areas and in cultures neurons, suggesting that **disruption of intracellular GSH homeostasis** is responsible for this injury [14].





Glutathione system

- A major endogeneous protective system is the glutathione redox cycle, GSH is a key component of the cellular defense cascade against injury caused by ROS [15].
- GSH was shown to modulate the level of ROS and participates in the cellular response to level of oxidative stress [15].
- GSH displays high intracellular concentrations in brain including hippocampus [16].
- Previous findings reported that GSH is essential for repair processes in hippocampal neurons exposed to oxidative damage induced by KA [17,18]



Aim of the study

- ✓ This study aimed to investigate the protective effects of glutathione level following KA administration in the rats' hippocampus on TH

- ✓ In the present experiment, we evaluate the amount of GSH, GSSG and GSH/GSSG ratio in the rats' hippocampus after the injection of KA and its effect on TH.

2. Methodology

Sprague Dawley male rats, n = 24

1. Control, n = 6

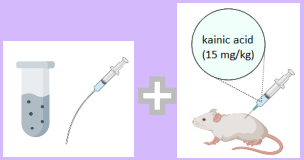
0.5 mL of distiller water

2. KA only, n = 6

0.5 mL of distiller water

3. KA + TH, n = 6

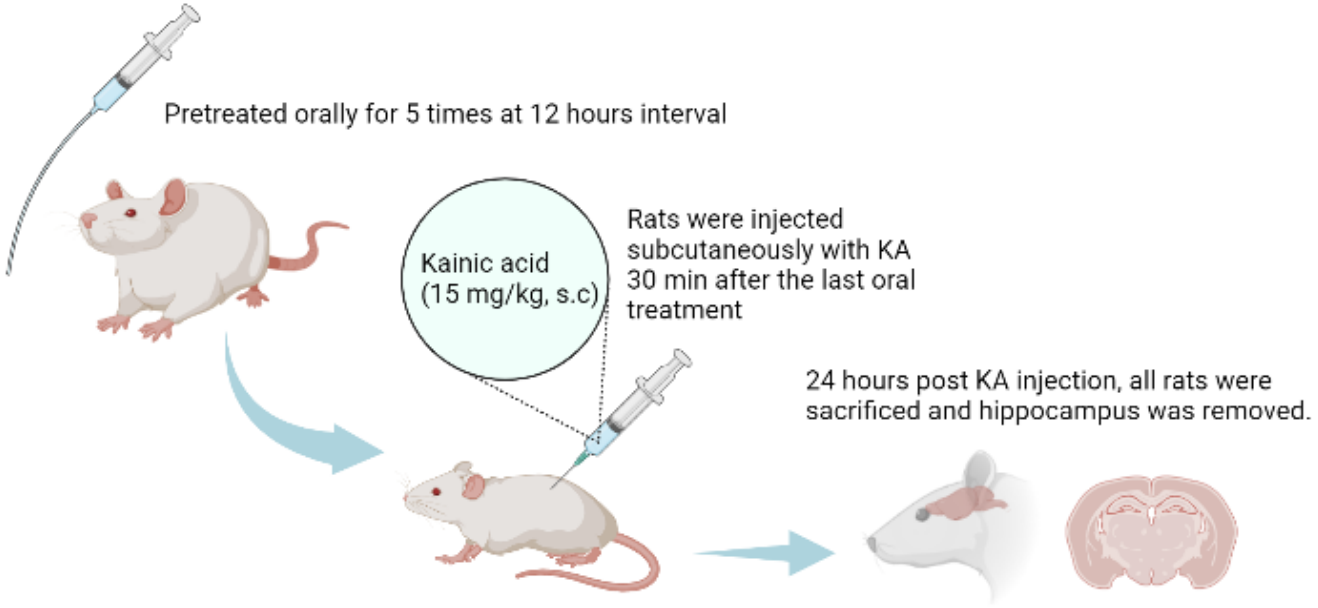
0.5 mL of Tualang honey (1 g/kg)



4. KA + TPM, n = 6

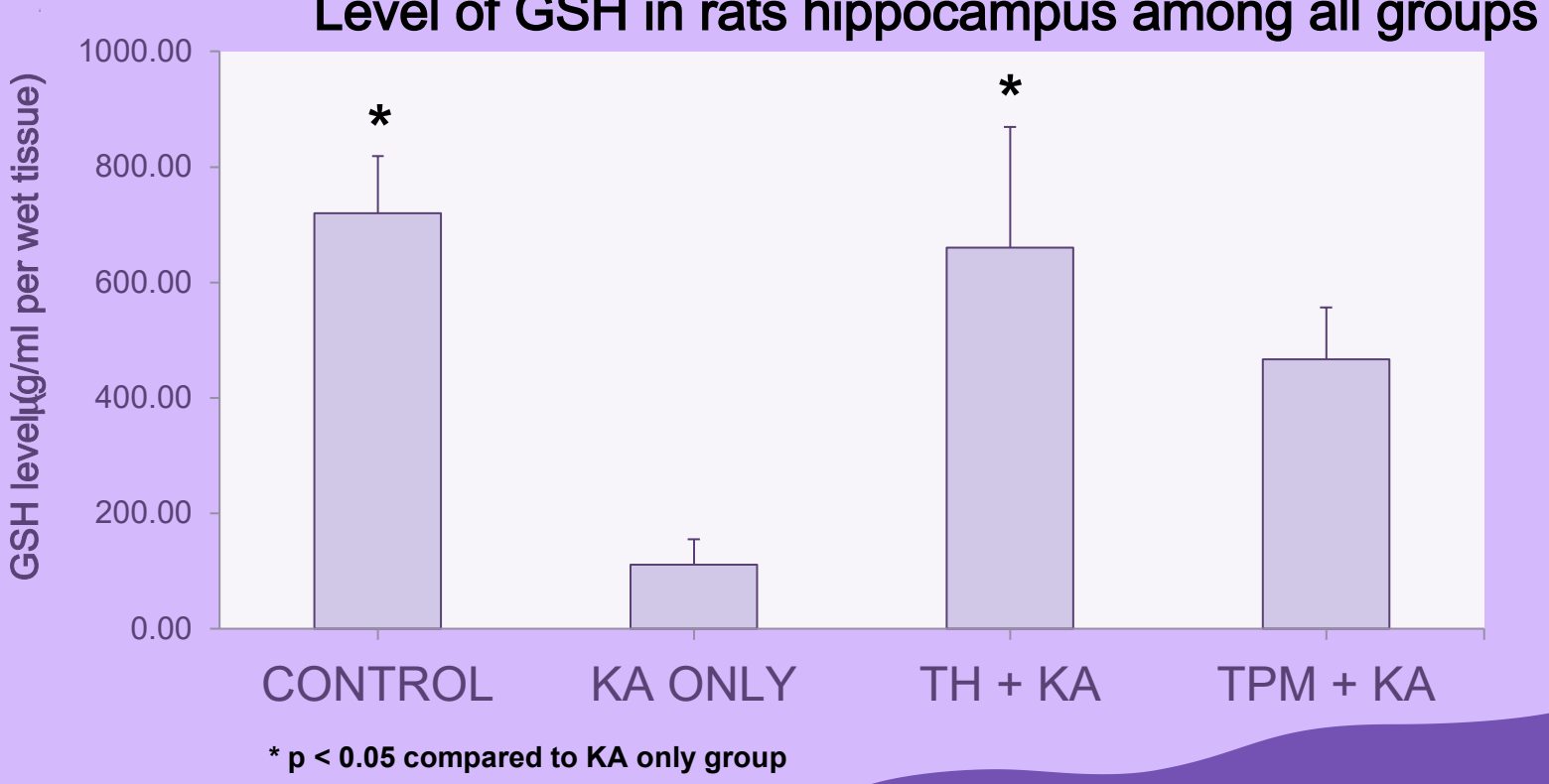
0.5 mL of Topiramate, antiepileptic drug (40 mg/kg)

Methodology



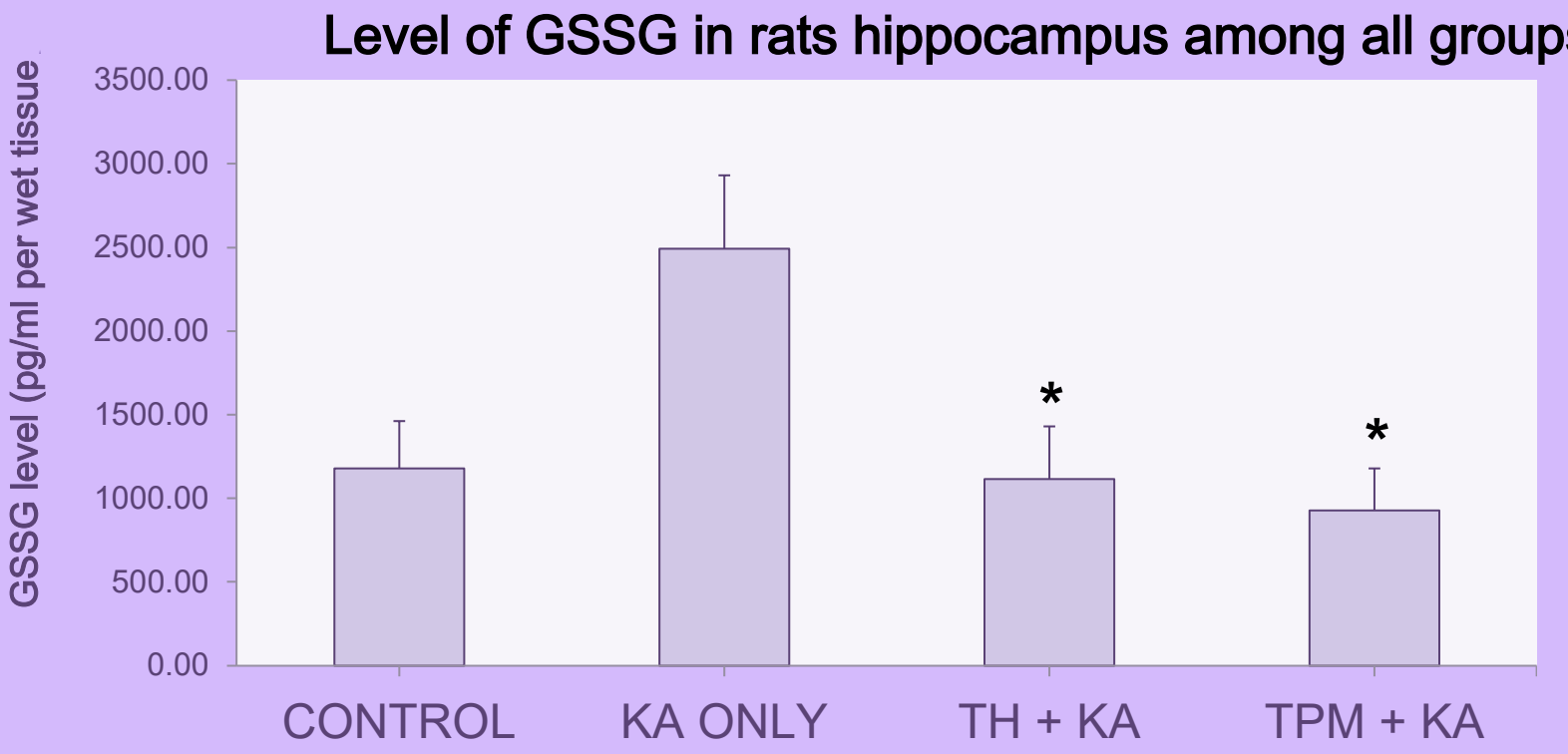
3. Results

Results



* p < 0.05 compared to KA only group

Results



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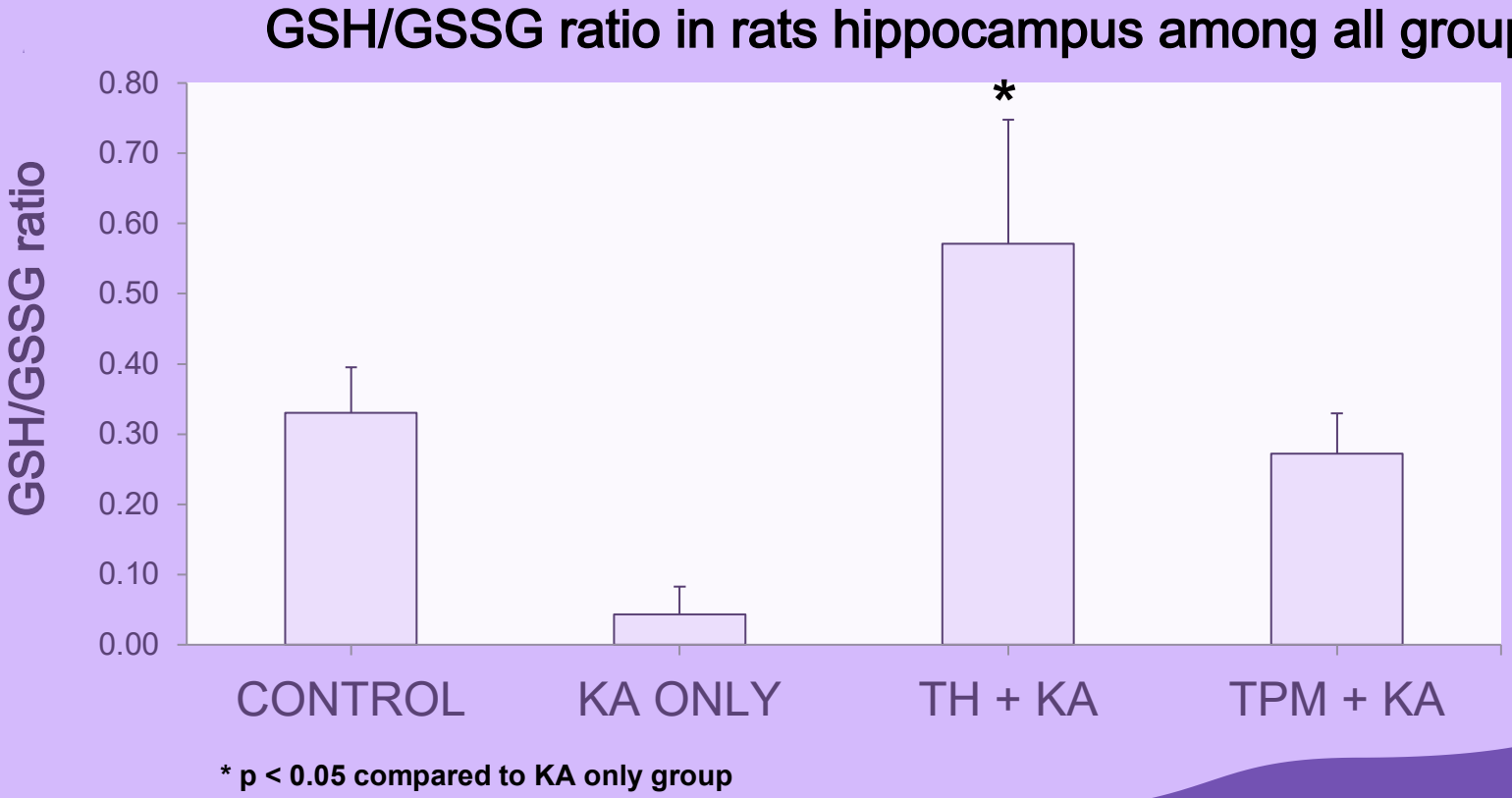
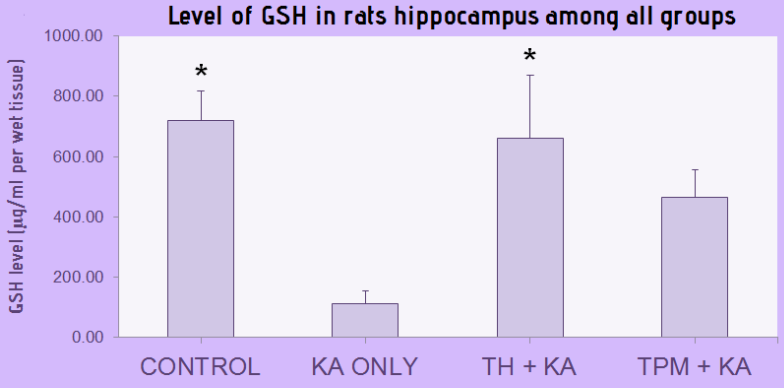
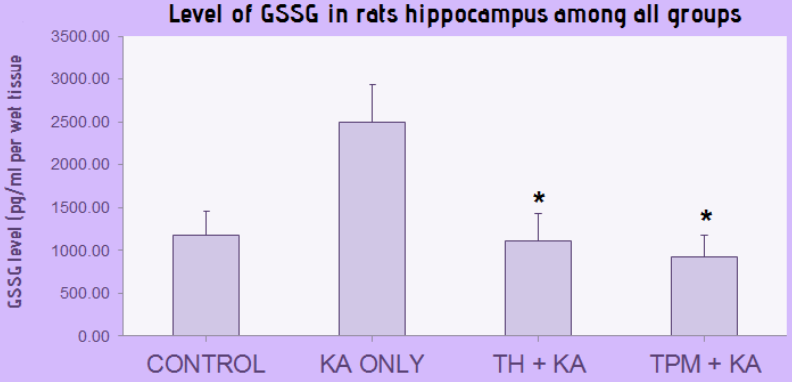
Results

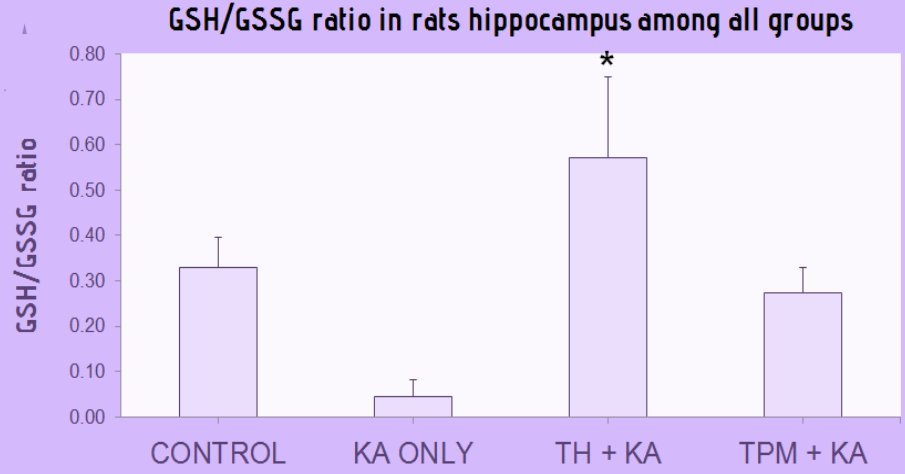


* p < 0.05 compared to KA only group

Results


Level of GSH in rats hippocampus among all groups
GSH level (µg/ml per wet tissue)
1000.00
800.00
600.00
400.00
200.00
0.00
*
*
CONTROL
KA ONLY
TH + KA
TPM + KA


Level of GSSG in rats hippocampus among all groups
GSSG level (pg/ml per wet tissue
3500.00
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
*
*
CONTROL
KA ONLY
TH + KA
TPM + KA


GSH/GSSG ratio in rats hippocampus among all groups
GSH/GSSG ratio
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
*
CONTROL
KA ONLY
TH + KA
TPM + KA

Discussion

- ✓ TH has been reported to contain various bioactive compounds, including **flavonoids** (catechin, kaempferol, naringenin, luteolin and apigenin) and **phenolic acids** (gallic, syringic, benzoic, trans-cinnamic, p-coumaric, and caffeic acids) that possess neuroprotective and antioxidant properties [19].
- ✓ For example, **apigenin** inhibits the KA-induced excitotoxicity of hippocampal cells in a dose dependent manner by quenching ROS and **inhibiting the depletion of GSH** levels [20].
- ✓ Besides that, **gallic acid** has been demonstrated to **decrease ROS production** and **lipid peroxidation** in KA-induced PC12 cells [21].
- ✓ It is postulated that the neuroprotective and antioxidant properties of TH may be exerted by flavonoids and possibly other polyphenolic compounds, as well as the synergistic effect of bioactive compounds [22].
- ✓ This findings was in similar with previous study which revealed that TH attenuated the decrease of GSH in the cerebellum and GSH/GSSG ratio in the brainstem after 24 h of KA administration [23].

Conclusions

- TH showed potential protective effects to prevent the **oxidative stress** related consequences by attenuating the **glutathione system** in the rats' hippocampus after KA administration.
- Further study need to be carried out to determine other biomarkers of oxidative stress to confirm the neuroprotective of TH in hippocampus after KA induction.

Acknowledgement

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Thanks!