UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

THE PROTECTIVE EFFECTS OF HONEY PROPOLIS ON KAINIC ACID MEDIATED EXCITOTOXICITY IN RAT BRAIN

PENYELIDIK

PROF. MADYA DR. MUMMEDY SWAMY

PENYELIDIK BERSAMA

PROF. MADYA DR. K.N.S SIRAJUDEEN
DR. ZULKARNAIN MUSTAPHA
EN. CHANDRAN GOVINDASAMY

2016

UNIVERSITI SAINS MALAYSIA

RESEARCH UNIVERSITY (RU) GRANT TECHNICAL REPORT

(Grant A/c. No. 1001/PPSP/813052)

MARCH 2016

Project Title

THE PROTECTIVE EFFECTS OF HONEY PROPOLIS ON KAINIC ACID MEDIATED EXCITOTOXICITY IN RAT BRAIN

Investigator: **Assoc. Prof. Dr. MUMMEDY SWAMY**

Co-Researchers: **Assoc. Prof.Dr. K.N.S. Sirajudeen**
Dr. Zulkarnain Mustapha and
En. Chandran Govindasamy

RESEARCH UNIVERSITY GRANT FINAL REPORT

UNIVERSITI SAINS MALAYSIA

RU GRANT
FINAL REPORT CHECKLIST

Please use this checklist to self-assess your report before submitting to RCMO.
Checklist should accompany the report.

NO.	ITEM	PLEASE CHECK (✓)		
		PI	JKPTJ	RCMO
1	Completed Final Report Form	√		✓
2	Project Financial Account Statement (e-Statement)	√		✓
3	Asset/Inventory Return Form *(Borang Penyerahan Aset/Inventori)*	√		✓
4	A copy of the publications/proceedings listed in Section D(ii) (Research Output)	√		✓
5	Comprehensive Technical Report	√		✓
6	Other supporting documents, if any	-		
7	Project Leader's Signature	√		✓
8	Endorsement of PTJ's Evaluation Committee	√		✓
9	Endorsement of Dean/ Director of PTJ's	√		✓

Project Code :
(for RCMO use only)

UNIVERSITI SAINS MALAYSIA

RU GRANT
FINAL REPORT FORM

Please email a softcopy of this report to rcmo@usm.my

A	**PROJECT DETAILS**
i	**Title of Research:** **THE PROTECTIVE EFFECTS OF HONEY PROPOLIS ON KAINIC ACID MEDIATED EXCITOTOXICITY IN RAT BRAIN**
ii	**Account Number:** **1001/PPSP/813052**
iii	**Name of Research Leader:** **Assoc. Prof. Dr. MUMMEDY SWAMY**
iv	**Name of Co-Researcher:** 1. Assoc. Prof. Dr. K.N.S. Sirajudeen 2. Dr. Zilkarnain Mustapha 3. Mr. Chanrdan Govindasamy
v	**Duration of this research:** **a) Start Date : 01/12/2011** **b) Completion Date : 30/11/2014** **c) Duration : 3 Years + 1 Year** **d) Revised Date (*if any*) : 31/12/2015**
B	**ABSTRACT OF RESEARCH**
	To understand the neuro-protective effects of propolis, the activities of nitric oxide synthase (NOS), glutamine synthetase (GS), caspase-3 and nitric oxide (NO), thiobarbituricacid reactive substances (TBARS), total antioxidant status (TAS) and tumor necrosis factor-α (TNF-α) levels along with the expression of neuronal NOS(nNOS), inducible NOS (iNOS), TNF-α, caspase-3 and GS were studied in cerebral cortex, cerebellum and brain stem in rats injected with kainic acid (KA) and in rats supplemented with propolis prior to excitotoxic injury with KA. Results of these studies clearly demonstrated that the propolis supplementation attenuated the NOS, caspase-3 activities, NO, and TNF-α concentration in KA mediated excitotoxicity. These studies were also demonstrated the restoration of GS activity and decreased oxidative stress by propolis in kainic acid mediated excitotoxicity. The expression studies of NOS isoforms did not show any change either with KA or propolis supplementation. Changes in expression of caspase-3, TNF-α and GS with KA administration were restored to normal with the prior supplementation of propolis. Hence the propolis can be a possible potential candidate (protective agent) against excitotoxicity and neurodegenerative disorders.

			Specific Objectives: 1. To determine the concentration of Nitrate/Nitrite, TBARS, TNF-α and TAS 2. To assay the activities of NOS, GS and Caspase-3 3. To determine the mRNA expression of NOS, GS, TNFα and Caspase-3 in different regions of brain of rats treated with KA alone and rats treated with honey propolis prior to KA administration.	Achieved Achieved Achieved

ii **Research Output**

a) **Publications in ISI Web of Science/Scopus**

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
1.	**Swamy, M.**, Wan Norlina Wan Azman, Dian Suhaili, K. N .S. Sirajudeen, Zulkarnain Mustapha and Chandran Govindasamy (2014) Restoration of glutamine synthetase activity, nitric oxide levels and amelioration of oxidative stress by propolis in kainic acid mediated excitotoxicity. Afr. J. Trad CAM. 11 (2), 458-463	Published
2.	**Swamy, M.**, Dian Suhaili, K. N .S. Sirajudeen, Zulkarnain Mustapha and Chandran Govindasamy (2014) Propolis ameliorates tumor necrosis factor-α, nitric oxide levels, caspase-3 and nitric oxide synthase activities in kainic acid mediated excitotoxicity in rat brain. Afr. J. Trad. CAM. 11 (5), 48 – 53	Published
3.	**Swamy,** M., Dian Suhaili, K. N .S. Sirajudeen, Zulkarnain Mustapha and Chandran Govindasamy (2015) Effect of propolis supplementation on nitric oxide synthase, glutamine synthetase, tumor necrosis factor-α and caspase-3 mRNA expression in kainic acid mediated excitotoxic rat brain. African Journal of Traditional, Complementary and Alternative Medicines	Under review

b) **Publications in Other Journals**

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
	Nil	

c) **Other Publications**
(book,chapters in book,monograph,magazine,etc.)

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
	Nil	

d) Conference Proceeding

No.	Conference (conference name,date,place)	Title of Abstract/Article	Level (International/National)
1	International Conference on Medical and Health Sciences (ICMHS) 22-24 May 2013, Kota Bharu, Kelantan	Effect of propolis on amelioration of oxidative stress in kainic acid mediated excitotoxicity in different regions of rat brain	International
2	International Symposium on Biological Engineering and Natural Sciences (ISBENS) 26-29 July 2013 Bangkok Thailand	Effect of propolis on restoration of Glutamine synthetase activity and Nitric oxide levels in kainic acid mediated excitotoxicity	International
3	International Conference on Natural Products 2014. 18th – 19th March 2014, Palm Garden Hotel, Putrajaya, Malaysia	Propol s attenuates the increased levels of TNF-α and nitric oxide in kainic acid mediated excitotoxic rat brain	International
4	International Conference on Natural Products 2014. 18th – 19th March 2014, Palm Garden Hotel, Putrajaya, Malaysia	Effect of propolis on caspase-3 activity and nitric oxide concentration in kain c acid induced excitotoxicity in rat bra n	International
5	Pharmacology & Physiology International Congress 2014. 22nd – 24th Aug 2014, Putra World Trade Centre, Kula Lumpur	Effect of propolis on nitric oxide synthase activity and nitric oxide concentration in kainic acid induced excitotoxic rat brain	International
6	Invited lecture at International Conference on Recent Advances in Research and Treatment of Human diseases & 4th Annual meeting of IABS. 9th – 11th January 2015, IICT, Hyderabad, India.	Antioxidant Propolis intervention in Kainic acid mediated excitotoxicity in rat bra n.	International
7	International Conference on Natural Products 2015, 24th – 25th March 2015, Double Tree by Hilton, Johor Bahru, Malaysia	Propol s ameliorates glutamine synthetase activity and expression in Kainic acid mediated excitotoxicity in rat bra n.	International
8	International Conference on Antioxidants & degenerative diseases 2015, 3rd – 4th June 2015, Hotel Istana, Kuala Lumpur, Malaysia	Effect of Propolis on Nitric oxide synthase Activity and Expression in Kainic acid induced Excitotoxic Rat brain.	International
9	29th Scientific meeting of Malaysian Society of Pharmacology and Physiology (MSPP). 24th – 25th Aug 2015, Setia City Convention Centre, Shah Alam, Malaysia	Effect of Propolis on TNF-α concentration and Expression in Kainic acid induced Excitotoxic Rat brain.	National
10	International Conference on Natural Products 2016, 15th – 17th March 2016, Permai Hotel, Kuala Terengganu, Kuala Terengganu, Malaysia.	Effect of Propolis on caspase-3 Expression and activity in Kainic acid induced Excitotoxic Rat brain.	International

 # Please attach a full copy of the publication/proceeding listed above

iii	Other Research Ouput/Impact From This Project *(patent, products, awards, copyright, external grant, networking, etc.)* Nil

E	HUMAN CAPITAL DEVELOPMENT

a) **Graduated Human Capital**

Student	Nationality (No.)		Name
	National	International	
PhD	-	-	-
M. Path. (Chemical Pathology)	1	-	Dr. Wan Norlina Wan Azman
Undergraduate	-	-	-

b) **On-going Human Capital**

Student	Nationality (No.)		Name
	National	International	
PhD	-	-	-
MSc	1	-	Ms. Dian Suhaili
Undergraduate	-	-	-

c) **Others Human Capital**

Student	Nationality (No.)		Name
	National	International	
Post Doctoral Fellow	-	-	-
Research Officer	-	-	-
Research Assistant	-	-	-
Others (.....)	-	-	-

F	COMPREHENSIVE TECHNICAL REPORT

Applicants are required to prepare a comprehensive technical report explaining the project. The following format should be used (this report must be attached separately):

- Introduction
- Objectives
- Methods
- Results
- Discussion

	• Conclusion and Suggestion • Acknowledgements • References

G	**PROBLEMS/CONSTRAINTS/CHALLENGES IF ANY**
	(Please provide issues arising from the project and how they were resolved) **Problem:** I have received 3rd yr allocation money on 17th Sept 2014 anly and there was a delay in molecular studies. **Resolved by:** One year extension of the project time was able to complete the work.
H	**RECOMMENDATION**
	(Please provide recommendations that can be used to improve the delivery of information; grant management, guidelines and policy, etc.) Nil

I	COMMENTS, IF ANY/ENDORSEMENT BY PTJ'S'RESEARCH COMMITTEE
	Kemajuan geran adalah baik. Objektif penyelidikan tercapai Penghasilan geran cemerlang. 2 pelajar siswazah dan 3 penerbitan dalam jurnal yg berimpak. Disarankan utk pembiayaan geran.

Appendix 1

UNIVERSITI SAINS MALAYSIA

BORANG PENYERAHAN ASET / INVENTORI

==========

A. BUTIR PENYELIDIK

1. NAMA PENYELIDIK : A. P. Dr. Mummedy Swamy.
2. NO STAF : 1327/10
3. PTJ : Sains Perubatan
4. KOD PROJEK :
5. TARIKH TAMAT PENYELIDIKAN : 31-12-2015.

B. MAKLUMAT ASET / INVENTORI : Nill

BIL	KETERANGAN ASET	NO HARTA	NO. SIRI	HARGA (RM)
	–	–	–	–

C. PERAKUAN PENYERAHAN

D. PERAKUAN PENERIMAAN

Saya telah memeriksa dan menyemak setiap alatan dan didapati :

☐ Lengkap
☐ Rosak
☐ Hilang : Nyatakan.....
☐ Lain-lain : Nyatakan .....

Diperakukan Oleh :

.....

Tandatangan
Pegawai Aset PTJ

Nama : .....
Tarikh : .....

***Nota :** Sesalinan borang yang telah lengkap perlulah dikemukakan kepada Unit Pengurusan Harta, Jabatan Bendahari dan Pejabat RCMO untuk tujuan rekod.

UNIVERSITI SAINS MALAYSIA

Tarikh Cetakan : 02/03/2016

JABATAN BENDAHARI
PENYATA PERBELANJAAN SEHINGGA 2 MAC 2016

Projek :

No. Akaun : 1001.PPSP.813052.

Vot	Nama Vot	Peruntukan Projek	Perbelanjaan Terkumpul Sehingga Thn Lalu	Baki Peruntukan Tahun Lalu	Peruntukan Thn Semasa	Jumlah Peruntukan Thn Semasa	Tanggungan Semasa	Bayaran Thn Semasa	Jum Belanja Thn Semasa	Baki Projek
111	GAJI	72,000.00	42,867.54	29,132.46	0.00	29,132.46	0.00	0.00	0.00	29,132.46
221	PERJALANAN DAN SARA HIDUP	7,000.00	16,493.75	-9,493.75	0.00	-9,493.75	0.00	0.00	0.00	-9,493.75
222	PENGANGKUTAN BARANG-BARANG	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
223	PERHUBUNGAN DAN UTILITI	0.00	25.97	-25.97	0.00	-25.97	0.00	0.00	0.00	-25.97
227	BEKALAN DAN BAHAN LAIN	131,400.00	111,220.18	20,179.82	0.00	20,179.82	0.00	25,683.20	25,683.20	-5,503.38
229	PERKHIDMATAN IKTISAS & HOSPITALITI	8,000.00	15,194.99	-7,194.99	0.00	-7,194.99	650.00	0.00	650.00	-7,844.99
	Jumlah	218,400.00	185,802.43	32,597.57	0.00	32,597.57	650.00	25,683.20	26,333.20	6,264.37

Penyata ini adalah cetakan komputer tiada tandatangan diperlukan

Penyata ini adalah dianggap tepat jika tiada maklumbalas dalam tempoh masa 14 hari dari tarikh penyata

COMPREHENSIVE TECHNICAL REPORT

TABLE OF CONTENTS

THE PROTECTIVE EFFECTS OF HONEY PROPOLIS ON KAINIC ACID MEDIATED EXCITOTOXICITY IN RAT BRAIN

Abstract

Glutamate receptor-mediated excitotoxicity is considered to be an important mechanism involved in various neurodegenerative diseases. Glutamine synthetase (GS) activity in the brain represents a key mechanism in regulation of excitatory neurotransmission. Increased nitric oxide (NO) levels, oxidative stress, neuronal inflammation and apoptosis have been proposed to be involved in excitotoxicity which plays a part in many neurodegenerative diseases. Honey bee propolis, with its antioxidant properties has been proposed to be protective on neurodegenerative disorders. To understand the neuro-protective effects of propolis, the activities of nitric oxide synthase (NOS), GS, caspase-3 and NO, thiobarbituricacid reactive substances (TBARS), total antioxidant status (TAS) and tumor necrosis factor-α (TNF-α) levels along with the expression of neuronal NOS(nNOS), inducible NOS (iNOS), TNF-α, caspase-3 and GS were studied in cerebral cortex, cerebellum and brain stem in rats injected with kainic acid (KA) and in rats supplemented with propolis prior to excitotoxic injury with KA. Male *Sprague-Dawley* rats were divided into control group, KA group, propolis group and propolis + KA group with six rats in each group. Propolis group and KA + propolis group were orally administered with propolis (150mg/kg body weight), five times every 12 hours. Control group received vehicle. KA group and KA+ propolis group were given subcutaneous injection of KA (15mg/kg body weight) and were sacrificed after 2 hrs along with other groups. The brain regions were separated, homogenized and used for estimation of TBARS, TAS and GS by spectrophotometrically and NO, NOS, TNF-α, caspase-3by commercial kits. RNA was extracted from the brain regions and converted to cDNA and in that nNOS, iNOS, GS, TNF-α, and

caspase-3 gene expression were carried out by RT-PCR. Results were analyzed by one-way ANOVA and reported as mean ± standard deviation and $p<0.05$ considered statistically significant. Results of these studies clearly demonstrated that the propolis supplementation attenuated the NOS, caspase-3 activities, NO, and TNF-α concentration in KA mediated excitotoxicity. These studies were also demonstrated the restoration of GS activity and decreased oxidative stress by propolis in kainic acid mediated excitotoxicity. The expression studies of NOS isoforms did not show any change either with KA or propolis supplementation. Changes in expression of caspase-3, TNF-α and GS with KA administration were restored to normal with the prior supplementation of propolis. Hence the propolis can be a possible potential candidate (protective agent) against excitotoxicity and neurodegenerative disorders.

Abstrak

Reseptor pengantara ketoksikan glutamate dianggap sebagai salah satu mekanisma yang terlibat dalam pelbagai penyakit kegagalan saraf. Aktiviti glutamine sintetase (GS) di dalam otak merupakan kunci bagi mekanisma kawal atur rangsangan penghantaran maklumat. Peningkatan tahap nitric oksida (NO), tekanan oksidatif, keradangan neuron dan apoptosis telah dicadangkan untuk terlibat dalam ketoksikan dimana ia memainkan peranan dalam kebanyakan penyakit kegagalan saraf. Madu lebah propolis, dengan sifat antioksidannya telah dicadangkan menjadi pelindung terhadap penyakit kegagalan saraf. Untuk memahami kesan perlindungan saraf oleh propolis, aktiviti-aktiviti nitrik oksida sintase (NOS), GS, caspase-3 dan NO, bahan reaktif asid thiobarbiturik (TBARS), jumlah status antioksidan (TAS), dan tahap tumor nekrosis factor-α (TNF-α) berserta dengan pengekspresan neuronal NOS (nNOS), inducible NOS (iNOS), TNF-α, caspase-3 dan GS telah dipelajari dalam kortek serebral, serebelum dan batang otak dalam tikus yang disuntik dengan asid kainik (KA) dan dalam tikus yang dtiambah dengan madu propolis sebelum kecederaan ketoksikan dengan KA. Tikus jantan *Sprague-Dawley* telah dibahagikan kepada kumpulan kawalan, kumpulan KA, kumpulan

propolis dan kumpulan propolis + KA dengan setiap kumpulan mempunyai enam ekor tikus. Kumpulan propolis dan kumpulan KA + propolis diberikan propolis dengan cara penyelenggaraan oral (150mg/kg jisim badan), 5 kali setiap 12 jam. Kumpulan kawalan menerima vehicle. Kumpulan KA dan kumpulan KA + propolis telah diberi suntikan subkutanius KA (15mg/kg jisim badan) dan dikorbankan selepas 2 jam berserta dengan kumpulan lain. Bahagian otak diasingkan, dihomogenasikan dan digunakan untuk anggaran TBARS, TAS dan GS secara spektrofotometrikal dan NO, NOS, TNF-α dan caspase-3 dengan cara kit komersial. RNA diekstrak dari bahagian-bahagian otak dan ditukarkan ke cDNA dan bagi pengekspresan gen nNOS, iNOS, GS, TNF-α dan caspase-3 dijalankan melalui RT-PCR. Keputusan dianalisa dengan one-way ANOVA dan dilaporkan sebagai purata ± sisihan piawai dan $p<0.05$ dianggap statistik yg signifikan. Keputusan kajian ini jelas menunjukkan penambahan propolis melemahkan NOS, aktivii caspase-3, NO, kepekatan TNF-α dalam KA pengantara ketoksikan. Kajian ini juga menunjukkan pemulihan aktiviti GS, dan penurunan tekanan oksidatif dalam KA pengantara ketoksikan oleh propolis. Pelajaran pengekspresan bagi NOS tidak menunjukkan sebarang perubahan sama ada dengan KA atau dengan penambahan propolis. Perubahan dalam pengekspresan caspase-3, TNF-α dan GS dengan penyelenggaraan KA dipulihkan ke normal sebelum penambahan propolis. Oleh itu, propolis sesuai dijadikan calon yang berpotensi sebagai (agen perlindungan) terhadap ketoksikan dan gangguan kegagalan saraf.

Keywords

Propolis, Kainic acid, Excitotoxicity, Nitric oxide, Nitric oxide synthase, Glutamine synthetats, Oxidative stress, TNF-α, Caspase-3

1. INTRODUCTION

Neuronal excitation involving the excitatory glutamate receptors is recognized as an important underlying mechanism in neurodegenerative disorders (Wong et al. 2005). Neurodegeneration is the progressive loss of structure and functions of neurons. Excitotoxicity has been considered to be a major pathological process of neuronal death in acute and chronic neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), temporal lobe epilepsy (TLE), amyotrophic lateral sclerosis (ALS) and hypoxia/ischemia (Doble, 1999). Excessive glutamate release is a major cause of cell death, possibly involving two athways. Firstly, excitotoxicity that occurs through the activation of glutamate receptors (Choi, 1988; Michael and Rothman1990), causing Ca2+ ion influx, with NMDA-mediated generation of Nitric oxide (NO), mitochondrial depolarisation, Na+ influx leading to an sustainable increase in ATP demond, microtubular depolarization, mitochondrial collapse and dendritic beading (Greenwood et al. 2007). Secondly, oxidative glutamate toxicity, that is mediated via a series of disturbances to the redox homeostasis of the cell (Murphy et al. 1989; Choi, 1992). The conversion of glutamate to glutamine by glutamine synthetase (GS), which takes place within the astrocytes represents a key mechanism in the regulation of excitatory neurotransmission under normal conditions as well as in injured brain(Szatkowski and Attwell1994). Kainic acid, a glutamate analogue is widely used to induce exitotoxicity in experimental animals, a model for temporal lobe epilepsy and a model for neurodegenerative disorders (Sperk 1994). Systemic administration of KA to rodents triggers a well characterized limbic seizures syndrome and selective neuronal degeneration attributed to the excitotoxic process (Doble, 1999; Lothman and Collins 1981; McKhann et al., 2003, Sperk, 1994). Effects of KA are mediated via activation of the kainite receptors that respond to the neurotransmitter glutamate and include the induction of inflammatory responses, production of cytokines and

neuronal death (Ullah et al., 2014). The molecular mechanisms by which KA induces excitotoxicity and cell death remain unclear; however, oxidative stress and the activation of proinflammatory cytokines are major contributors (Wong et al., 2005). Thus, anti-oxidant and anti-inflammatory treatment could attenuate or prevent KA-induced neurodegeneration (Zhang and Zhu, 2011). Both in vitro and in vivo studies demonstrate that KA induces cell death via accumulation of intracellular calcium, which stimulates ROS production and mitochondrial dysfunction, thereby leading to neuronal cell death (Wong et al., 2005; Hilton et al., 2005). There is evidence for activation of calpain- and caspases-induced neural apoptosis following KA exposure (Smialowska et al., 2011). Besides oxidative stress and intracellular calcium overload, KA can activate molecular mechanisms leading to depletion of neuronal energy stores and thereby activating the alternative cell death pathways (Choi, 1987; Culmsee et al., 2001; McCullough, 2005). Our earlier studies demonstrated increased oxidative stress and increased production of NO along with increased activity of NO synthase and decreased activity of GS in KA mediated excitotoxicity (Swamy et al 2009, 2011).

In recent decades, there is an emerging trend to search for natural resources to combat against neurodegenerative diseases. Many studies have tested or reported the protective effect of natural products against KA-induced excitotoxicity *in vivo* and *in vitro* models (Han et al., 2012; Hou, 2011; Huang *et al.*, 2012; Shukitt-Hale et al., 2008; Zeng et al., 2013). Honey bee propolis has been widely used as a folk medicine and proposed to be protective on neurodegenerative disorders (Ha et al., 2010; Kwon et al., 2004). It has been shown to have broad biological activities, which are principally attributed to the presence of flavonoids (Isla et al., 2001) and caffeic acid phenyl ester (CAPE) (Natarajan et al., 1996). The prevailing opinion is that the broad biological activities of flavonoids and CAPE are related, in part, to their anti-inflammatory and anti oxidant actions (Isla et al., 2001; Natarajan et al., 1996). Therefore we hypothesized that honey propolis will protect the brain from KA mediated excitotoxicity and neurodegeneration.

1.1. OBJECTIVES

General objective:

To study the protective effects of honey propolis on Kainic acid (KA) mediated

excitotoxicity in different regions rat brain

Specific Objectives:

1. To determine the concentration of Nitrate/Nitrite, TBARS and TAS
2. To assay the activities of NOS, GS and Caspase-3
3. To determine the mRNA expression of NOS, GS, TNFα and Caspase-3

in different regions of brain of rats treated with KA alone and rats treated with honey propolis prior to KA administration.

Each of the brain regions was weighed and used for the preparation of homogenates in 0.05M phosphate buffer pH 7.3 and were used for different enzyme assays, nitrate/nitrite estimation, TBARS, TAS and TNFα estimation. For mRNA expression studies tissue was placed in corresponding medium.

Enzyme assay

Glutamine synthetase: GS activity was assayed by the method Rowe et al. (1970) as described by Swamy et al (2011a). Assay mixture consisting of 0.4 ml of imidazole-HCl buffer (pH 7.2), 0.1 ml of 0.2 M magnesium chloride, 0.1 ml of 0.25 M 2-mercaptoethanol, 0.1 ml of 0.1 M ATP, 0.1 ml of 0.5 M glutamate, 0.1 ml of 1 M hydroxyl amine (pH 7.2) and 0.1 ml of 10% homogenates were incubated for 15 min at 37 ^{0}C. At the end of incubation 1.5 ml of ferric chloride reagent (0.37 M $FeCl_3$, 0.67 M HCl & 0.2 M TCA) was added to terminate the reaction and to initiate the colour development. Controls received homogenate after the addition of ferric chloride reagent. A reagent blank was prepared by omitting homogenate from the assay mixture. After centrifugation, the absorbency in the supernatant was measured at 535 nm. The amount of γ-glutamyl hydroxamate formed was calculated using that one μmol of γ-glutamyl hydroxamate gives an OD of 0.34 at 535 nm. Enzyme activity expressed as μmol of γ-glutamyl hydroxamate formed/g wet weight of tissue/hour.

Nitric oxide synthase: NOS activity was estimated by the method of Yui et al. (1991) as described by Swamy et al. (2011a), in which the stable end products, NOx, were estimated using the Nitric Oxide Synthase assay Kit from Calbiochem, U.S.A. (Catalogue Number 482702). The optical density was measured at 540 nm using VersaMax ELISA, Microplate Reader. NOS activity was expressed as nano mole NOx /g wet tissue/ hour

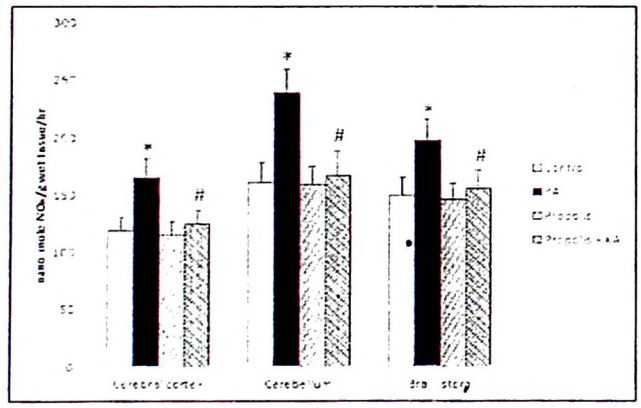
Caspase-3: Caspase-3 activity was assayed using Caspase-3/CPP32 colorimetric assay kit from BioVision Research Products, Milpitas, California, USA. The optical density was measured

Results

The activity of NOS, NO concentration and GS activity

The activity of NOS and NO concentration were increased significantly ($p<0.001$) in all the three brain regions tested in KA group compared to control group, but the increased activity of NOS and NO concentration by KA were prevented by prior supplementation of propolis (Figure 1 and Figure 2). There were no significant differences in the activity of NOS and NO concentration between control and propolis as well as propolis + KA group (Figure 1 and Figure 2). GS activity was decreased significantly ($p<0.001$) in all the three brain regions in KA group compared to control group and propolis + KA group indicating propolis treatment was preventing ($p<0.001$ in CB; $p<0.01$ in CC and BS) the GS activity decrease observed by KA treatment. There was no significant difference in GS activity between control and propolis as well as propolis + KA group propolis + KA group (Figure 3).

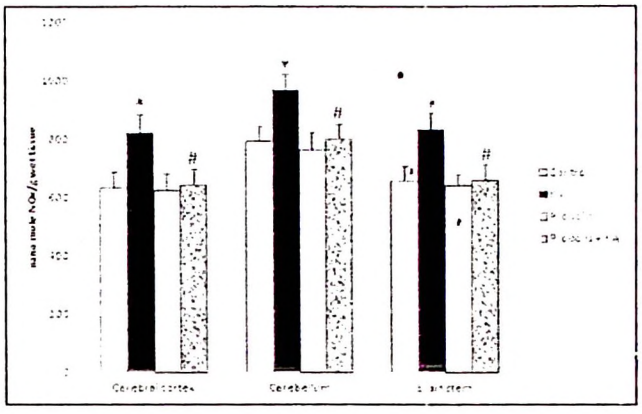
Figure 1: The activity of NOS in KA mediated excitotoxicity and propolis supplementation



Values are mean ± SD from 6 rats

*$p<0.001$ versus control group; #$p<0.001$ versus KA group

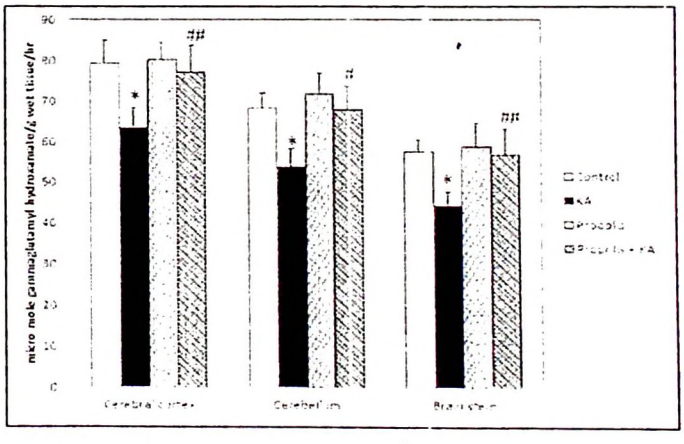
Figure 2: The Concentration of NOx in KA mediated excitotoxicity and propolis supplementation



Values are mean ± SD from 6 rats

*p<0.001 versus control group; #p<0.001 versus KA group

Figure 3: The activity of GS in KA mediated excitotoxicity and propolis supplementation



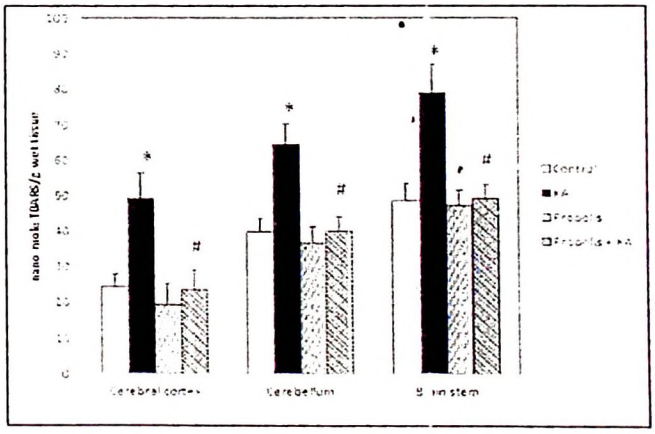
Values are mean ± SD from 6 rats

*p<0.001 versus control group; #p<0.001, ##p<0.01 versus KA group

The concentration of TBARS and TAS

The concentration of TBARS was increased significantly ($p<0.001$) in all the three brain regions tested in KA group compared to control group, but the increase of TBARS concentration by KA was prevented ($p<0.001$) (Figure 4) by prior supplementation with propolis (propolis + KA group). There was no significant difference in TBARS concentration between control and propolis as well as propolis + KA group (Figure 4).The concentration of TAS was decreased significantly ($p<0.001$) in KA group compared to control and propolis + KA group indicating the depletion of TAS concentration by KA was prevented ($p<0.001$ in CB; $p<0.01$ in CC and BS) (Figure 5) by supplementation of propolis (propolis + KA group). There was no significant difference in TAS concentration between control and propolis as well as propolis + KA group propolis + KA group (Figure 5).

Figure 4: The Concentration of TBARS in KA mediated excitotoxicity and propolis supplementation



Values are mean ± SD from 6 rats

*$p<0.001$ versus control group; #$p<0.001$ versus KA group