The Protective Effect of Propolis on Norepinephrine, Dopamine and 5-Hydroxytryptamine Content in Thalamus-Hypothalamus and Cerebellum of Endotoxin-Intoxicated Adult Male Albino Rats

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Abstract: The present study was undertaken to investigate the effect of endotoxin (ET) on monoamines content in the thalamus-hypothalamus and the cerebellum of adult male albino rats and whether propolis (prop) can protect the brain during neuro-inflammation induced by ET. Seventy eight rats weighing 100-150g were divided into four groups. The first served as control group (6 rats) and were received a daily intraperitoneal (i.p) injection of saline solution (0.9% NaCl) for 15 consecutive days. The second group (24 rats); were received an i.p injection of 0.9% NaCl for 15 consecutive days, then received i.p injection of ET (1mg/kg/day) at the 16th day of experiment for 4 repeated days. The third group (24 rats); were received an i.p injection of prop (150mg/kg/day) for 19 consecutive days. The fourth group (24 rats); were administered prop by the same route as mentioned in the third group; then, the rats were received ET as described in the second group. Animals of all groups were decapitated 2 hours post-treatment at 16th, 17th, 18th and 19th days of experiment. ET treated-rats group showed a sharp decrease in norepinehrine (NE), dopamine (DA) and 5-hydroxytryptamine (serotonin, 5-HT) content in both thalamus-hypothalamus and cerebellum at all treatment days versus the control group, while administration of prop followed by ET injection was found to enhance monoamine levels significantly in both selected brain regions if compared to ET-treated group. The present results indicate the harmful neurotoxic effect of ET on the brain, while prop was found to inhibit the sharp decline in NE, DA and 5-HT in both investigated regions. This may reflect the protective property of prop as an anti-inflammatory natural product.
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1. Introduction

Endotoxin is an inflammatory lipopolysaccharide (LPS) molecule from gram-negative bacteria that are ubiquitous in the door environment **(Thorne *et al.*, 2005)**. It is biologically effective even in lowest concentrations **(Todar, 2002)**. Bacterial DNA and LPS are potent activators of immune cells such as monocytes and macrophages which contribute to systemic inflammatory responses syndrome **(Hong *et al.*, 2004)**. Some key physiological responses of ET shock in man include fever, reduced blood pressure, metabolic acidosis and tissue damage was indicated after ET exposure **(Hodgson, 2006)**. **Coskun *et al.* (2005)** reported that ET injection produces renal damage, increased lipid peroxidation and decreased antioxidant enzyme activity. Moreover, several reports indicated that ET was accompanied by significant changes in neurotransmitter levels in different parts of the CNS **(Koulchitsky *et al.*, 2000; Dunn, 2005)** and causes neuronal damage **(Gao *et al.*, 2003).**

Propolis is a sticky resin that seeps from the buds of some trees and oozes from the bark of other trees. The bees gather prop and carry it home in their pollen baskets. They blend it with wax flakes secreted from special glands on their abdomens **(Gregory, 2002)**. It was found that prop is efficient against conditions caused by bacteria, viruses or different fungi. It cures many diseases because it is a special natural substance with strong effect. Moreover, prop possesses many biological activities such as antitumor, antioxidant, antimicrobial, anti-inflammatory and also immunomodulator **(Mani *et al.*, 2006).**

Tandon *et al.* (2006) indicated that NE, DA and 5-HT are biogenic amines that serve as neurotransmitters in a number of nerve pathways in the brain. NE is secreted by many neurons to help control the overall activity and mood of the mind, while DA acts as an inhibitory transmitter; whereas 5-TH acts as an inhibitor of pain pathways in the cord and its actions in the higher regions of the nervous system are believed to control the mood.

The present work aims to shed light on the disturbances in NE, DA and 5-HT after ET injection and the protective role of prop as a natural anti-inflammatory substance in lowering ET harmful neurotoxic effect on these amines in the thalamus-hypothalamus and cerebellum of adult male albino rats.

2. Materials and Methods:

Drug used:

Endotoxin was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Propolis was purchased from Holding Company for Biological Products & Vaccines (VACSERA), Giza, Egypt.

Experimental animals:

Adult male albino rats (*Rattus norvegicus*) weighing 100-150g were obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Giza, Egypt). After acclimatization for a period of one week, animals were divided into four groups (6 rats per group) and housed in wire bottomed cages in a room under standard conditions of illumination with a 12-hours light-dark cycle. They were provided with water and a balanced diet *ad libitum*. All animals received care in compliance with the Egyptian rules for animal protection.

Rats were randomized and divided into four groups, one control of six rats and three treated groups each of 24 rats, each of which was subdivided into 4 subgroups six rats at each as follows:

Group 1: (The control group) in which animals received a daily i.p injection of normal saline solution (0.9%NaCl) for 15 consecutive days.

Group 2: (ET group), rats were received daily i.p normal saline solution (0.9%NaCl) for 15 consecutive days then followed by i.p injection of ET (1mg/Kg body weight) at the 16^{th}, 17^{th}, 18^{th} and 19^{th} days of the experiment.

Group 3: (Prop group), rats were received an aqueous extract of 10% prop (1gm of prop soaked for several days in 9ml distilled water then filtered through a clean and very fine filter paper **(Krell, 1996)**. Animals of this group were received a daily i.p injection of prop (150mg/kg body weight) for 19 consecutive days.

Group 4: (prop and ET group), rats were received a daily i.p injection of prop as shown in group 3, while the treatment of ET in combination with prop injection was carried on the 16^{th},17^{th}, 18^{th} and 19^{th} days of experiment as described in the second group.

Methods:

Animals of all groups were killed by fast decapitation two hours after the last dose, at the 16^{th}, 17^{th}, 18^{th} and 19^{th} days of the experiment. The brains were carefully removed, blotted and frozen. Dissection was performed on ice-cold glass plate for the separation of the thalamus-hypothalamus and cerebellum regions from the brain within one minute according to the method of **Glowinski & Iversen (1966).**

Estimation of NE, DA and 5-HT was undertaken according to the flourimetric method described by **Ciarlone (1978).**

Statistical analysis:

Results were expressed as the mean ± standard error of the mean. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, **Duncan's test (1955)** was used as post hoc test according to the statistical package program (SPSS version 8). Percentage difference representing the percent of variation with respect to the control group was also calculated.

3-Results

The present data concerning the influence of i.p injection of ET and/or prop on monoamines content are presented as follows: Table (1) to (6) revealed that, ET injection caused a sharp and highly significant decrease at $p<0.001$ in NE, DA and 5-HT levels in the thalamus-hypothalamus and cerebellum of adult male albino rats at all time intervals of experiment as compared to control group.

The illustrated data in tables (1) and (2) concerning prop group indicated an increase in the NE content in thalamus-hypothalamus and cerebellum at 16^{th}, 17^{th}, 18^{th} and 19^{th} days of treatment with respect to control group. The increase in NE content in the thalamus-hypothalamus was of a significant change at $p<0.05$, while in the cerebellum was of a significant change at $p<0.001$ with a percentage difference of 31.6%, 26.3%, 42.1% and 26.3%, respectively. On the other hand, tables (3-6) revealed that, prop treated-rats showed a significant decrease in DA and 5-HT contents in both examined regions at all time intervals of the experiment with respect to control group.

As depicted from tables (1) to (6), injection of ET to prop-treated rats showed significant decreases in NE, DA, and 5-HT levels at $p<0.001$ in thalamus-hypothalamus and cerebellum at the 16^{th}, 17^{th}, 18^{th} and 19^{th} days of treatment except at the 17^{th} day, the NE levels in the thalamus-hypothalamus showed a slight and non significant decrease as compared to control group. On the other hand, the levels of NE, DA and 5-HT indicated a significant enhancement in both thalamus-hypothalamus and cerebellum versus ET-treated group at all days of the experiment indicating the protective role of prop against ET effect. Meanwhile, prop & ET-treated rats group revealed a highly significant decrease in NE, DA and 5-HT levels at $p<0.001$ in both the thalamus-hypothalamus and cerebellum at the 16^{th}, 17^{th}, 18^{th} and 19^{th} days of treatment versus prop-treated group.

Table (1): Effect of intraperitoneal injection of endotoxin (1mg/kg b.wt.) and/or propolis (150mg/kg b.wt.) on norepinephrine content (µg/g tissue) in thalamus-hypothalamus of adult male albino rats at the 16th, 17th, 18th and 19th days of treatment; the number of animals was 6 in each group. Data are expressed as mean ± SE.

Experimental Days	Experimental Groups (Mean ± SE)			
	Control	ET	Prop	Prop & ET
16th	0.35±0.01	0.16±0.01 (-54.3)a**	0.38±0.01 (8.6)a	0.28±0.003 (-20.0)a**b**c**
17th		0.24±0.01 (-31.4)a**	0.40±0.01 (14.3)a	0.34±0.01 (-2.9)b**c**
18th		0.08±0.003 (-77.1)a**	0.37±0.01 (5.7)a	0.25±0.10 (-28.6)a**b**c**
19th		0.17±0.01 (-51.4)a**	0.37±0.02 (5.7)a	0.19±0.003 (-45.7)a**bc**

a: Significant change at $p < 0.05$ with respect to control group. b: Significant change at $p < 0.05$ with respect to endotoxin group.
c: Significant change at $p < 0.05$ with respect to propolis group. * - ** changes between $p < 0.01$ - $p < 0.001$.
( ): % difference with respect to control value

Table (2): Effect of intraperitoneal injection of endotoxin (1mg/kg b.wt.) and/or propolis (150mg/kg b.wt.) on norepinephrine content (µg/g tissue) in cerebellum of adult male albino rats at the 16th, 17th, 18th and 19th days of treatment; the number of animals was 6 in each group. Data are expressed as mean ± SE.

Experimental Days	**Experimental Groups** (Mean ± SE)			
	Control	**ET**	**Prop**	**Prop & ET**
16th	0.19±0.01	0.05±0.003 (-73.7)a**	0.25±0.01 (31.6)a**	0.07±0.003 (-63.2)a**b**c**
17th		0.07±0.001 (-63.2)a**	0.24±0.01 (26.3)a**	0.097±0.003 (-48.9)a**b**c**
18th		0.03±0.002 (-84.2)a**	0.27±0.01 (42.1)a**	0.06±0.001 (-68.4)a**b**c**
19th		0.10±0.01 (-47.4)a**	0.24±0.01 (26.3)a**	0.15±0.005 (-21.1)a**b**c**

a: Significant change at $p < 0.05$ with respect to control group. b: Significant change at $p < 0.05$ with respect to endotoxin group.
c: Significant change at $p < 0.05$ with respect to propolis group. * - ** changes between $p < 0.01$ - $p < 0.001$.
( ): % difference with respect to control value

Table (3): Effect of intraperitoneal injection of endotoxin (1mg/kg b.wt.) and/or propolis (150mg/kg b.wt.) on dopamine content (µg/g tissue) in thalamus-hypothalamus of adult male albino rats at the 16th, 17th, 18th and 19th days of treatment; the number of animals was 6 in each group. Data are expressed as mean ± SE.

Experimental Days	**Experimental Groups** (Mean ± SE)			
	Control	**ET**	**Prop**	**Prop & ET**
16th	3.84±0.01	1.77±0.03 (-53.9)a**	3.53±0.02 (-8.1)a**	3.02±0.03 (-21.4)a**b**c**
17th		2.86±0.05 (-25.5)a**	3.54±0.01 (-7.8)a**	3.45±0.02 (-10.2)a**b**c**
18th		1.28±0.02 (-66.7)a**	3.51±0.01 (-8.6)a**	2.10±0.03 (-45.3)a**b*c**
19th		2.53±0.02 (-34.1)a**	3.53±0.02 (-8.1)a**	2.97±0.08 (-22.7)a**b**c**

a: Significant change at $p < 0.05$ with respect to control group. b: Significant change at $p < 0.05$ with respect to endotoxin group.
c: Significant change at $p < 0.05$ with respect to propolis group. * - ** changes between $p < 0.01$ - $p < 0.001$.
( ): % difference with respect to control value

Table (4): Effect of intraperitoneal injection of endotoxin (1mg/kg b.wt.) and/or propolis (150mg/kg b.wt.) on dopamine content (µg/g tissue) in cerebellum of adult male albino rats at the 16th, 17th, 18th and 19th days of treatment; the number of animals was 6 in each group. Data are expressed as mean ± SE.

Experimental Days	**Experimental Groups** (Mean ± SE)			
	Control	**ET**	**Prop**	**Prop & ET**
16th	2.90±0.03	1.05±0.01 (-63.8)a**	2.57±0.02 (-11.4)a**	1.27±0.01 (-56.3)a**b*c**
17th		1.70±0.02 (-41.4)a**	2.54±0.02 (-12.4)a**	2.06±0.01 (-29.0)a**b**c**
18th		0.60±0.02 (-79.3)a**	2.60±0.01 (-10.3)a**	1.09±0.01 (-62.4)a**b**c**
19th		0.96±0.02 (-66.9)a**	2.58±0.01 (-11.0)a**	1.76±0.01 (-39.3)a**b**c**

a: Significant change at $p < 0.05$ with respect to control group. b: Significant change at $p < 0.05$ with respect to endotoxin group.
c: Significant change at $p < 0.05$ with respect to propolis group. * - ** changes between $p < 0.01$ - $p < 0.001$.
( ): % difference with respect to control value

Table (5): Effect of intraperitoneal injection of endotoxin (1mg/kg b.wt.) and/or propolis (150mg/kg b.wt.) on serotonin content (µg/g tissue) in thalamus-hypothalamus of adult male albino rats at the 16th, 17th, 18th and 19th days of treatment; the number of animals was 6 in each group. Data are expressed as mean ± SE.

Experimental Days	Experimental Groups (Mean ± SE)			
	Control	ET	Prop	Prop & ET
16th	5.78±0.03	1.63±0.02 (-71.8)^{a**}	5.19±0.07 (-10.2)^{a**}	2.39±0.02 (-58.7)^{a**b**c**}
17th		2.58±0.02 (-55.4)^{a**}	5.28±0.02 (-8.7)^{a**}	3.23±0.02 (-44.1)^{a**b**c**}
18th		1.22±0.02 (-78.9)^{a**}	5.19±0.07 (-10.2)^{a**}	1.73±0.06 (-70.1)^{a**b*c**}
19th		2.36±0.02 (-59.2)^{a**}	5.14±0.09 (-11.1)^{a**}	3.06±0.02 (-47.1)^{a**b**c**}

a: Significant change at $p < 0.05$ with respect to control group. b: Significant change at $p < 0.05$ with respect to endotoxin group.
c: Significant change at $p < 0.05$ with respect to propolis group. * - ** changes between $p < 0.01$ - $p < 0.001$.
( ): % difference with respect to control value

Table (6): Effect of intraperitoneal injection of endotoxin (1mg/kg b.wt.) and/or propolis (150mg/kg b.wt.) on serotonin content (µg/g tissue) in cerebellum of adult male albino rats at the 16th, 17th, 18th and 19th days of treatment; the number of animals was 6 in each group. Data are expressed as mean ± SE.

Experimental Days	Experimental Groups (Mean ± SE)			
	Control	ET	Prop	Prop & ET
16th	3.04±0.01	0.51±0.01 (-83.2)^{a**}	2.81±0.03 (-7.6)^{a}	0.62±0.02 (-79.6)^{a**bc**}
17th		0.95±0.02 (-68.8)^{a**}	2.77±0.02 (-8.9)^{a}	1.52±0.02 (-50.0)^{a**b**c**}
18th		0.49±0.01 (-83.9)^{a**}	2.86±0.02 (-5.9)^{a}	0.60±0.02 (-80.3)^{a**b*c**}
19th		1.26±0.01 (-58.6)^{a**}	2.79±0.01 (-8.2)^{a}	1.61±0.02 (-47.0)^{a**b**c**}

a: Significant change at $p < 0.05$ with respect to control group. b: Significant change at $p < 0.05$ with respect to endotoxin group.
c: Significant change at $p < 0.05$ with respect to propolis group. * - ** changes between $p < 0.01$ - $p < 0.001$.
( ): % difference with respect to control value

4. Discussion

Results of the present study revealed sharp decreases in the levels of NE, DA and 5-HT in the thalamus-hypothalamus and cerebellum of ET-treated rats at all time intervals of the experiment, if compared to control group. These results are in agreement with **Masana *et al.* (1990); Dunn (1992) and Koulchitsky *et al.* (2000)**.

Masana *et al.* (1990) indicated elevated levels of NE at 4, 8 and 24 hour following LPS injection (20µg/rat) with an increased level of DA metabolite in the striatum, hypothalamus and medulla oblongata**. Dunn (1992)**, found that i.p injection of LPS activated cerebral catecholamine and the hypothalamo-pituitary adrenocortical axis; increased mouse brain concentration of NE catabolite, 3-methoxy,4- hydroxyphenyl ethylene glycol; increased the DA catabolite, 3,4-dihydroxy phenyl acetic acid; increased the 5-HT catabolite, 5-hydroxy-indole acetic acid and tryptophan in all brain regions examined. Moreover**, Koulchitsky *et al.* (2000)** reported significant changes in 5-HT, NE, histamine, kininis and gamma aminobutyric acid catabolism in different parts of CNS at the initial phase reaction of ET treatment.

As an environmental inflammation-inducer LPS has been shown as a potent microglia activator and neurotoxin *in vitro* and *in vivo* studies **(Arimoto & Bing, 2003).** LPS elicits responses similar to that after interleukin-1, either used peripherally or centrally as it induces NE release in the brain most markedly in the hypothalamus, accompanied by small changes in brain DA **(Dunn 2005)**. The author added that both bacterial and viral infections induce hypothalamic pituitary axis activation and also increased brain NE, 5-HT metabolism and brain tryptophan. LPS has been reported to stimulate the secretion of pro inflammatory mediators and cytokines **(Erickson & Banks, 2011)** indicating the induction of neuro-inflammation. Neuro-inflammation is important in the pathogenesis and progression of Alzheimer disease as it causes memory impairments and disturbance in monoamine release **(Lee *et al.*, 2012).** Moreover, microglia, the major resident immune cells in the brain, switch to an activated phenotype in response to pathogen invasion or tissue damage and thereby promote an inflammatory response including release of free radicals, cytokines and lipid metabolites **(Glass *et al.*, 2010),** which in turn trigger a neuro-degenerative

cascade via neuro-inflammation **(Qiuntanilla *et al*., 2004**).

Recently, **Kaneko *et al*. (2012)** examined the regulation of activated microglia through their cell death and survival pathways. The authors suggested that long-lived microglia resulting from exposure to the optimal dose of LPS may play critical role in the progression of neurodegeneration. **Pocock and Kettenmann (2007**) indicated that, catecholamine NE is a classical neurotransmitter with suggested immunomodulatory properties released by neurons into the synaptic cleft and may exert effects on glia cells. NE may also have an immune suppressive role as it attenuates LPS-induced microglial production of tumor necrosis factor (TNF-α) interleukin-6 and nitric oxide and reduces microglial-induced neuronal cell death **(Madrigal *et al*., 2005).**

Lately, **Dunn (2005)**, reported that many of the cytokines (most notably interleukin-1 and interleukin-6 act via the brain, resulting in the activation of the corticotropin releasing factor containing neurons in the hypothalamic paraventricular nucleus. The author suggested the ability of interleukin-1 and interleukin-6 to elevate circulating glucocorticoids as it is critical for the survival of the organism. It has become evident that there may exist a cross-talk between the autonomic nervous system and the immune system during inflammation **(Sternberg, 2006)**.

Systemic LPS treatment was found to mitigate methamphetamine-induced striatal DA and 3, 4-dihydroxyphenyl acetic acid depletions in a dose-dependent manner **(Lin *et al*., 2007a)**. Moreover, **Jovanovic *et al*. (1997)** reported that loss of substantia nigra neurons resulted in reduced synthesis and release of DA from nerve terminals. It has been reported that, either using single midbrain slice culture or triple culture, LPS induces dopaminergic neuronal loss which was accompanied by increased microglia activation and nitric oxide production **(Xing *et al*., 2010).** Increasing evidence has suggested that oxidative stress **(Elkon *et al*., 2004)** and neuro-inflammation **(Herrera *et al*., 2005)** are involved in the process of dopaminergic neuronal loss. Inflammation and oxidative stress-mediated by microglia has been known to be a significant pathological feature of Parkinson disease **(Jenner and Olnaw, 1998).** Microglia activation has been suggested to play an important role in initiating and/or amplifying neuronal injury since not only the substantia nigra has an extreme high density of resting microglia but also reactive microglia are found in close proximity to the damaged nigral neurons in the brain of Parkinson disease patients **(Le *et al*., 2001).**

A variety of deleterious pro-inflammatory factors and cytokines released from activated microglia trigger the dopaminergic neurodegeneration **(Le *et al*., 2001)** and in turn the dying neurons can release microglia-activating molecules **(Zhang *et al*., 2011)** forming a vicious degenerative cycle. **Lin *et al*. (2007b)** indicated that, the microglia cell mediated the neurotoxin 6-hydroxy dopamine (6-OHDA)-induced toxicity in human dopaminergic neuroblastoma cells. In addition, **Mazzio *et al*. (2003)** indicated that LPS injection leads to accumulation of DA metabolites which contribute to degeneration of dopaminergic neurons.

Recently, **Fan *et al*. (2011)** found that although the neonatal LPS-induced neurobehavioral impairment was spontaneously recoverable, the LPS exposure induced persistent injury to the dopaminergic system and chronic inflammation may represent the existence of silent neurotoxicity. The authors further suggested that the compromised mitochondrial function might contribute partially, to silent neurotoxicity, which was first proposed by **Reuhl (1991)** as persistent morphological and/or biochemical injury which remains unapparent unless unmasked by experimental or natural processes. It is likely that rat exposure to LPS at different stages of dopaminergic development may contribute to the different pattern in dopaminergic system injury seen in **Fan *et al*. (2011)** as compared to that reported by **Ling *et al*. (2006)** who observed a progressive dopamine neuron loss following supra-nigral LPS infusion in prenatal rats.

Gaykema and Goehler (2009) suggested that the sickness behavior that ensues as a result of ET injection and inflammation is characterized by diminished arousal and motivation and reduced appetite may emerge as a result of diminished activity of the hypothalamic orexin system which could be a prime candidate for the neurotoxicity driven by peripheral innate immune activation. Moreover, **Almeida *et al*. (2006)** supported that LPS-induced cold-seeking response is mediated by neuronal bodies located in the dorso-medial hypothalamus and neural fibers passing through the paraventricular nucleus in the hypothalamus, which may explain ET-shock due to LPS exposure.

On the other hand, the sharp decreases in 5-HT level in thalamus-hypothalamus and cerebellum after ET injection in the present study are in agreement with the results of **Cho *et al*. (2000)** who supported that LPS activates the TNF-α which exerts pronounced effects on 5-HT metabolism in most brain regions. Furthermore, LPS produced an increase in the extracellular concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus, which was paralleled by a significant

decline in behavioral activity and a marked increase in extracellular corticosterone levels **(Linthorst *et al*., 1995).**

Moreover, i.p injection of LPS increased mouse brain concentrations of the 5-HT catabolite, 5-HIAA at all the examined brain regions **(Dunn**, **1992).** Also, the authors indicated that stressful treatments with ET have been shown previously to elevate brain concentrations of tryptophan, and concluded that activation of the sympathetic nervous system is responsible for the stress-related increase in brain tryptophan, probably by enabling increased brain tryptophan uptake. The increase in brain tryptophan appears to be necessary to sustain the increased 5-HT catabolism to 5-HIAA**.** Recently, **Del Angel-Meza *et al*. (2011)** showed that tryptophan which plays an important role in immune system, protein synthesis and melatonin production is a potent endogenous free radical scavenger antioxidant, has a protective effect in the oxidative damage in the model of endotoxic shock in the breeding nursling-induced by systemic administration of LPS (20mg/kg) acting as a scavenger of free radicals. The authors proposed that tryptophan is an innocuous protector agent in the endotoxic shock process.

The present results indicated that Prop & ET-treated rats showed a significant decrease in monoamines level as compared to control group, while showed a significant increase in NE, DA and 5-HT content if compared to ET-treated rats. The present results are in agreement with the results documented by **Noelker *et al*. (2005); Shimazawa *et al*. (2005); Ma *et al*. (2006); Cengiz *et al*. (2007) and Vauzour *et al*. (2007)**.

Propolis is one of the few natural remedies that maintained its popularity over a long period of time. The composition of prop is highly variable depending on the local plant and is reported to contain approximately 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% other substances (minerals) **(Cohen *et al.*, 2004)**. Furthermore, prop contains a mixture of biologically active chemicals including terpenes, cinnamic acid, caffeic acid and their esters, amino acids and flavonoids **(Volpert & Elstner, 1993)**. Caffeic acid phenethyl ester (CAPE) was reported to have antitumor **(Huang *et al*., 1996)** anti-inflammatory **(Michaluort *et al*., 1999)** and antioxidant properties **(Ahn *et al*., 2004).** Pinocembrin is the most abundant flavonoids in prop, and has been proven to have antioxidant, antibacterial and anti-inflammatory property **(Gao *et al*., 2008)**.

The antioxidant function of prop may be an underlying mechanism by which prop protects against neuronal damage. **Shimazawa *et al*. (2005)** indicated that prop significantly inhibited neurotoxicity and protected mouse forebrain against oxidative stress (lipid peroxidation). The authors postulated that the scavenging free radicals and inhibition of oxidative stress may be partly responsible for prop neuroprotective function against *in vitro* cell death and *in vivo* focal cerebral ischemia. Also, the antioxidant function of prop may be an underlying mechanism by which prop protects against neuronal damage. Moreover, **Noelker *et al*. (2005)** reported that prop derivatives, in particular CAPE may have a neuroprotective effect on neuronal cells and may also be a promising drug candidate to be taken into the *in vivo* models of Parkinson disease.

Ma *et al*. (2006) reported that pretreatment with CAPE prevented 6-OHDA induced neurotoxicity. The authors suggested that CAPE blocks 6-OHDA induced neuronal death possibly by increasing both antioxidation and neuroprotection effects**.** Moreover**, Cengiz *et al*. (2007)** observed that CAPE prevented vacuolization, an indication of brain edema, and showed that pretreatment with a single i.p injection of CAPE reduced the structural changes in the brain. **Vauzour *et al*.(2007)** indicated that CAPE contains a catechol moiety which inhibited tyrosinase-induced oxidation thus yielding cysteinyl polyphenol adducts, a mechanism by which polyphenols exert protection against neuronal injury relevant to neurodegenerative diseases. Furthermore, **Wang *et al*. (2009)** indicated that CAPE inhibited cytokine and chemokine-production by monocyte-denderitic cells which might be related to the nuclear factor signaling pathway during allergic disorders.

It could be concluded from this study that i.p injection of ET induced a dramatic change in monoaminergic system in the thalamus-hypothalamus and cerebellum of white male albino rats. It has been proven that the treatment of prop has antibacterial and anti-inflammatory properties which could abolish the disturbance in nervous system induced by ET treatment.

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5. References

1. **Ahn, M. R.; Kumazawa, S.; Hamasaka, T.; Bang, K.S. and Nakayama, T. (2004):** Antioxidant activity and constituents of propolis collected in various areas of Korea. J. Agric. Food Chem.; 52: 7286-92.
2. **Almeida, M. C.; Steiner, A. A.; Branco, L. G. and Romanovsky, A. (2006):** Neural substrate of cold-seeking behavior in Endotoxin shock. Plos One; 1(1): e1.
3. **Arimoto, T. and Bing, G. (2003)** Up-regulation of inducible nitric oxide synthase in the substantia nigra by

lipopolysaccharide causes microglial activation and neurodegeneration. Neurobiol. Dis.; 12: 35-45.

4. **Cengiz, N.; Colakoglu, N.; Kavakli, A.; Sahna, E.; Parlakpinar, H. and Acet, A. (2007):** Effects of caffeic acid phenethyl ester on cerebral cortex: structural changes resulting from middle cerebral artery ischemia reperfusion. *Clin. Neuropathol.; 26(2): 80-84.*
5. **Ciarlone, A. E. (1978):** Further modification of a fluorimetric method for analyzing brain amines. Microchem. J.; 23; 9-12.
6. **Cho, I; Tsunoda, M and Sharma, R. P. (2000):** Effects of endotoxin and tumor necrosis factor on regional brain neurotransmitters in mice. *Nat. Tox;7(5):187–195.*
7. **Cohen, H. A.; Varsano, I.; Kahan, E.; Sarrell, E. M. and Uziel,Y. (2004):** Effectiveness of an herbal preparation containing Echinacea, propolis, and vitamin C in preventing respiratory tract infections in children: a randomized, double-blind, placebo-controlled, multicenter study. Arch Pediatr. Adolesc. Med.; 158: 217-221.
8. **Coskun, O.; Armuteu, F.; Kanter, M. and Kuzey, G. M. (2005**): Protection of endotoxin-induced oxidative renal tissue damage of rats by vitamin E or/and EGb 761 treatment. J. Appl.Toxicol.; 25(1): 8-12.
9. **Del Angel-Meza, A. R.; Dávalos-Marín, A. J.; Ontiveros-Martinez, L. L.; Ortiz, G. G.; Beas-Zarate, C.; Chaparro-Huerta, V.; Torres-Mendoza, B. M. and Bitzer-Quintero, O. K. (2011):** Protective effects of tryptophan on neuro-inflammation in rats after administering lipopolysaccharide. Biomed. Pharmacother; 65(3): 215-219.
10. **Duncan, D.B. (1955):** Multiple F -test. *Biometrics; 11:1-42.*
11. **Dunn, A. J. (2005):** Handbook of Stress and the Brain, Part 2: Stress: Integrative and Clinical Aspects Techniques in the Behavioral and Neural Sciences**;** Chapter 2.2 Cytokine activation of the hypothalamo-pituitary-adrenal axis. 15(2): 7-17. **Dunn, A. J. (1992):** Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: comparison with interleukin-1. Am. Soc. Pharmacol. Expr. Therap.; 261(3): 964-969.
12. **Elkon, H.; Melamed, E. and Offen, D. (2004):** Oxidative stress, induced by 6-hydroxydopamine, reduces proteasome activities in PC12 cells: implications for the pathogenesis of Parkinson's disease. J. Mol. Neurosci.; 24: 387-400.
13. **Erickson, M. A. and Banks, W. A. (2011):** Cytokine and chemokine responses in serum and brain after single and repeated injections of lipopolysaccharide: Multiplex quantification with path analysis. Brain Behav. Immun.; 25: 1637-1648.
14. **Fan, L. W; Tien, L. T.; Zheng, B. ; Pang, Y.; Lin, R. C.; Simpson, K.; Ma, T.; Rhodes**, **P. G. and CaiZ. (2011):** Dopaminergic neuronal injury in the adult rat brain following neonatal exposure to lipopolysaccharide and the silent neurotoxicity. Brain Behav. Immun.; 25(2): 286-297.
15. **Gao, H.; Liu, B. and Hong, J. S. (2003):** Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. *J. Neurosci.; 23: 6181-6187.*
16. **Gao, M.; Zhang, W.; Liu, Q.; Hu, J.; Liu, G. and Du, G. (2008):** Pinocembrin prevents glutamate-induced apoptosis in SH-SY5Y neuronal cells via decrease of bax/bcl-2 ratio. *Europ. J. of Pharmacol.; 591:73–79.*
17. **Gaykema, R. P. A. and Goehler, L. E. (2009):** Lipopolysaccharide challenge-induced suppression of Fos in hypothalamic orexin neurons: their potential role in sickness behavior. *Brain Behav Immun* 23(7): 926–930.
18. **Glass, C. K.; Saijo, K.; Winner, B.; Marchetto, M. C. And Gage, F. H. (2010):** Mechanisms underlying inflammation in neurodegeneration. Cell; 140: 918-934.
19. **Glowinski, L. J. and Iversen, L. L. (1966):** Regional studies of catecholamines in the rat brain. I. Disposition of Ha-noradrenaline, Ha-dopamine and Ha-dopa in various regions of the brain. J. Neurochem.; 13: 655-669.
20. **Gregory, S. R. (2002):** Comparison of Silver Sulfadiazine to Propolis in Second-Degree Burn Treatment. *J. of Alternat. and Complement. Med.; 8(1):77-83.*
21. **Herrera, A. J.; Tomas-Camardiel, M.; Venero, J. L.; Cano, J. and Machado, A. (2005):** Inflammatory process as a determinant factor for the degeneration of substantia nigra dopaminergic neurons. J. Neural. Transm.; 112: 111-119.
22. **Hodgson, J. C. (2006):** Endotoxin and mammalian host responses during experimental disease. J. Comp. Path.; 135:157-175.
23. **Hong, Z.; Jiang, Z.; Liangxi, W.; Guofu, D.; Ping, L. and Yongling, L. (2004):** Chloroquin protects mice from challenge with CPG ODN and LPS by decreasing proinflammatory cytokine release. Int. Immunopharmacol.; 4(2): 223-34.
24. **Huang, M. T.; Ma, W.; Yen, P.; Xie, J. G.; Han, J.; Frenkel, K.; Grunberger, D. and Conney, A. H. (1996):** Inhibitory effects of caffeic acid phenethyl ester (CAPE) on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion in mouse skin and the synthesis of DNA, RNA and protein in Hela cells. Carcinog.; 17: 761-765.
25. **Jenner, P. and Olnaw, C. W. (1998):** Understanding cell death in Parkinson's disease. Ann. Neurol.; 44: S72–S84.
26. **Jovanovic, M.; Malicevic, Z.; Jovicic, A.; Djukic, M.; Ninkovic, M.; Jelenkovic, A. and Mrsulja, B. (1997):** Selective sensitivity of the striatum to oxidative stress. Vojnosanit. Pregl; 54: 33- 43.
27. **Kaneko, Y. S.; Nakashima, A.; Mori, K.; Nagatsu, T.; Nagatsu, I. and Ota, A. (2012):** Microglial activation in neuroinflammation: implications for the etiology of neurodegeneration. Neurodegener. 10(1-4):100-103.
28. **Koulchitsky, S. V.; Galina, K.; Tropnikova, A. and Mironova, G. P. (2000**): Central pool of serotonin and tail-flick latency during two phases of biphasic fever in rats. Proc. Natl. Sci. Counc. ROC (B); 24(3): 123-128.
29. **Krell, R. (1996):** Propolis. Value- added products from beekeeping. The Chief ed., FAO, Viale delle Terme di Caracalla, Italy; 124(5).
30. **Le, W.; Rowe, D.; Xie, W.; Ortiz, I.; He, Y. and Appel, S. H. (2001):** Microglial activation and dopaminergic cell injury: an *in vitro* model relevant to Parkinson's disease. J. Neurosci.; 21: 8447-8455.
31. **Lee, Y. J.; Choi, D. y.; Choi, I. S.; Kim, K. H. ; Kim, Y. H.; Kim, H. M.; Lee, K. and Cho, S. (2012):** Inhibitory effect of 4-*O*-methylhonokiol on lipopolysaccharide-induced neuroinflammation, amyloidogenesis and memory impairment via inhibition of nuclear factor-kappaB *in vitro* and *in vivo* models. J. Neuroinflammation; 9: 35.
32. **Lin, Y. C.; Kou, Y. M.; Liao, P. C.; Cherng, C. G.; Su, S. W. and Yu, L. (2007a):** Attenuation of

methamphetamine-induced nigrostriatal dopaminergic neurotoxicity in mice by lipopolysaccharide pretreatment. Chin. J. Physiol; 50(2): 51-56.

33. **Lin, Y. C.; Uang, H. W.; Lin, R. J.; Chen, I. J. and Lo, Y. C. (2007b):** Neuroprotective effects of glycerol nonivamide against microglia- like cell 6-hydroxydopamine-induced neurotoxicity in SH-SY5Y human dopaminergic neuroblastoma cells. J. Pharmacol. Exp. Ther.; 323(3): 877-887.
34. **Ling, Z.; Zhu, Y.; Tong, C.; Snyder, J. A.; Lipton, J. W. and Carvey, P. M. (2006):** Progressive dopamine neuron loss following supra-nigral lipopolysaccharide (LPS) infusion into rats exposed to LPS prenatally. Exp. Neurol.; 199: 499-512.
35. **Linthorst, A. C. E.; Flachskamm, C.; Preuss, P. M.; Holsboer, F. And Reul, J. M. H. M. (1995):** Effect of Bacterial Endotoxin and Interleukin-IP on Hippocampal Serotonergic Neurotransmission, Behavioral Activity, and Free Corticosterone Levels: An *in vivo* Microdialysis Study. *J. of Neurosci.; 7(74): 2920-2934*.
36. **Ma, Z.; Wei, X.; Fontanilla, C.; Noelker, C.; Dodel, R.; Hampel, H. and Du, Y. (2006):** Caffeic acid phenethyl ester blocks free radical generation and 6-hydroxydopamine-induced neurotoxicity. Life Sci.; 79(13): 1307-1311.
37. **Madrigal, J. L.; Feinstein, D. L. and Dello Russo, C. (2005):** Norepinephrine protects cortical neurons against microglial-induced cell death. J. Neurosci. Res. 81: 390-396.
38. **Mani, F.; Damasceno, H. C.; Novelli, E. L.; Martins, E. A. and Sforcin, J. M. (2006):** Propolis: Effect of different concentrations, extracts and intake period on seric biochemical variables. J. Ethnopharmacol.; 105(1-2): 95-98.
39. **Masana, M. I.; Heyes, M. P. and Mefford, I. N. (1990):** Indomethacin prevents increased catecholamine turnover in rat brain following systemic endotoxin challenge. Prog. Neuropsychopharmacol. Biol. Psychiatry.; 14(4): 609-621.
40. **Mazzio, E.; Becker, A. and Soliman, K. F.(2003):** Inflammation and inducible nitric oxide synthase have no effect on monoamine oxidase activity in glioma cells. Biochem. Pharmacol.; 65(10): 1719-1727.
41. **Michaluart, P; Masferrer,J. L.; Carothers, A. M.; Subbaramaiah, K.; Zweifel, B. S.; Koboldt, C.; Mestre, J. R.; Grunberger, D.; Sacks, P. G.; Tanabe, T. and Dannenber, A. J. (1999):** Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. Cancer Res.; 59(10): 2347-2352.
42. **Noelker, C.; Bacher, M.; Gocke, P.; Wei, X.; Klockgether, T.; Du, Y.; and Dodel, R. (2005):** The flavanoide caffeic acid phenethyl ester blocks 6-hydroxydopamine-induced neurotoxicity. Neurosci. Lett.; 383(1-2): 39-43.
43. **Pocock, J. M. and Kettenmann, H. (2007):** Neurotransmitter receptors on microglia. Trends Neurosci.; 30: 527-535.
44. **Quintanilla, R. A.; Orellana, D. I.; Gonzalez-Billault, C. and Maccioni, R. B. (2004):** Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. Exp. Cell Res.; 295: 245-257.
45. **Reuhl, K. R. (1991):** Delayed expression of neurotoxicity: the problem of silent damage. Neurotoxicol.; 12: 341-346.
46. **Shimazawa, M.; Chikamatsu, S.; Morimoto, N.; Mishima, S.; Nagai, H. and Hara, H. (2005):** Neuroprotection by Brazilian Green Propolis against *In vitro and In vivo* Ischemic Neuronal Damage. Evid Based Complement Alternat. Med.; 2(2): 201-207.
47. **Sternberg, E. M. (2006):** Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. Nat. Rev. Immunol.; 6: 318-328.
48. **Tandon, A. A.; Bhalla, P.; Nagpaul, J. P. and Dhawan, D. K. (2006):** Effect of lithium on rat cerebrum under different dietary protein regimens. *Drug and Chem. Toxicol.;29(4):333-344*.
49. **Thorne, P. S.; Kulhánková, K.; Yin, M.; Cohn, R.; Arbes, S. J. and Zeldin, D. C. (2005)**: Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in United States housing. Am. J. Respir. Crit. Care Med.; 172(11): 1371-1377.
50. **Todar, K. (2002):** Mechanisms of bacterial pathogenicity: Endotoxins. Todar's Online Textbook of Bacteriology, by Dr. Kenneth Todar, University of Wisconsin Department of Bacteriology, 1550 Linden Drive Madison, Wisconsin 53706.
51. **Vauzour, D.; Vafeiadou, K.; Rice-Evans, C; Williams, R. J. and Spencer, J. P. (2007**): Activation of pro-survival Akt and ERK1/2 signalling pathways underlie the anti-apoptotic effects of flavanones in cortical neurons. J. Neurochem.; 103(4): 1355-1367.
52. **Volpert, R. and Elstner, E. F. (1993):** Biochemical activities of propolis extracts. I. Standardization and antioxidative properties of ethanolic and aqueous derivatives. Z. Naturforsch. C.; 48 (11-12): 851-857.
53. **Wang, L. C.; Lin, Y. L.; Liang, Y. C.; Yang, Y. C.; Lee, J. H.; Yu, H. H.; Wu, W. M. and Chiang B. L. (2009):** The effect of caffeic acid phenethyl ester on the functions of human monocyte-derived dendritic cells. B. M. C. Immunol.; 10: 39.
54. **Xing, B.; Xin, T.; Zhao, L.; Hunter, R. L.; Chen, Y. and Bing, G. (2010**): Glial cell line-derived neurotrophic factor protects midbrain dopaminergic neurons against lipopolysaccharide neurotoxicity. J. Neuroimmunol.; 225(1-2): 43-51.
55. **Zhang, W.; Phillips, K.; Wielgus, A. R.; Liu, J.; Albertini, A.; Zucca, F. A.; Faust, R.; Qian, S. Y.; Miller, D. S.; Chignell, C. F.; Wilson, B.; Jackson-Lewis, V.; Przedborski, S.; Joset, D.; Loike, J.; Hong, J. S.; Sulzer, D. and Zecca, L. (2011):** Neuromelanin Activates Microglia and Induces Degeneration of Dopaminergic Neurons: Implications for Progression of Parkinson's Disease. Neurotox. Res.; (1): 63-72.

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