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Full Length Research Paper

Possible protective effect of propolis against lead-induced neurotoxicity in animal model

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Lead (Pb) is widespread toxic metals found in the environment and potential danger to human health. This study investigated the possible protective roles of propolis against Pb-induced neurotoxicity. Forty male adult (Swiss albino rats) rats were divided into four groups: Group-I {Control: 1 ml distilled water intraperitoneal (i.p.)}; group-II, received propolis at a dose of 50 mg/kg/day daily orally (p.o.); group-III, (Pb treatment: 1 mg/kg i.p.) and group-IV (propolis and Pb treatment: 50 mg propolis/kg p.o. and Pb (1 mg/kg i.p.) daily for four weeks. Activity level of acetylcholinesterase (AChE activity), lipid peroxidation (MDA), protein carbonyl content (PCC), sulphhydryl proteins (P-SH), vitamin C, vitamin E, NADH-cytochrome C reductase, succinate dehydrogenase (SDH) and cytochrome oxidase activities were examined in different groups. Results showed that co-administration of propolis with Pb inhibited Pb-induced neurological toxicity as indicated by normalization of AChE activity, inhibition of brain MDA and PCC formation. In addition, propolis protects the mitochondrial NADH-cytochrome C reductase, SDH and cytochrome C activities from Pb-induced amelioration. Furthermore, propolis increased brain vitamin C, vitamin E and P-SH levels in rat's brain both in group treated by propolis alone and group treated by propolis and Pb. It can be concluded that propolis has beneficial effects and could be able to antagonize Pb-induced neurotoxicity.

Key words: Propolis, lead, neurotoxicity, oxidative stress.

INTRODUCTION

Lead (Pb) is widespread toxic metals found in the environment and potential danger to human health due to its multifaceted action with a broad range of physiological and biochemical dysfunctions (Gurer and Ercal, 2000). It is known that Lead has detrimental effects on the central and peripheral nervous systems (Adonaylo and Oteiza, 1999). Exposure to low-levels of Lead leads to the behavioral abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and in also reported in laboratory animals (Ruff et al., 1996).

Biochemical and molecular mechanisms of Lead toxicity is poorly understood (Shalan et al., 2005). Various mechanisms were suggested to explain them: Inhibition of the calcium-pump, inactivation of P450 enzymes and nervous tissues demyelinisation (Yücebilgiç et al., 2003), cholinergic dysfunction, glutamate receptor alteration, and enhanced oxidative stress. Bolin et al. (2006) and Ribarov and Bochev (1982) also suggested that the lead is causative factors in neurotoxicity.

It had been previously shown that oxidative stress because of decreased antioxidant function might be the main mechanism involved in brain neurotoxicity induced by Pb-exposure (Wang et al., 2006). Recently, propolis, naturally occurring antioxidant, has been reported as a powerful ROS scavenger in rat (Ozguner et al., 2005). Therefore, the present study aimed to investigate the effect of co-treatment with antioxidant propolis against Pb-induced neurotoxicity.

Propolis or bee glue is a resinous product, collected by honey bees from plant exudates and contains more than 160 constituents (Greenaway et al., 1991). It had been shown to have broad biological activities which are principally attributed to the presence of flavonoids (major component: Rutin, quercetin and galangin) and caffeic acid phenethyl ester (CAPE) (Isla et al., 2001). Propolis having flavonoids which are responsible for many of biological and pharmacological activities including anticancer (Matsuno, 1995), anti-inflammatory (Wang et

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al., 1993), antimicrobial (Koo et al., 2000), and antioxidant effects (Mohammadzadeh et al., 2007). Propolis has neuroprotective effects, but these effects partly mediated via antioxidant effects (Nakajima et al., 2007).

Recently, propolis had been reported as a powerful scavenger of reactive oxygen species (ROS) (Ozguner et al., 2005) and few studies conducted these effects on central nervous system functions only in the animal models. Therefore, the present study attention is focused on whether the administration of propolis to rats could protect against Lead-induced-neurotoxicity.

MATERIALS AND METHODS

Animals

The adult Swiss albino rats (nos=40: 6 month old-200 to 250 grams weight) raised from a stock obtained from the Veterinary Medicine Faculty, Zagazig University, Egypt, were reared in local animal house conditions, when they were fed on pellet feed (El-Nasr Chemical Company, Cairo, Egypt) *water ad libitum*. The Animal Ethical Committee approved the design of the experiments, and the guidelines of the National Institutes of Health (NIH). The animals were housed under controlled environmental conditions (12 h light/dark cycle) at a temperature of 25±1°C and humidity of 60±5% and left for two weeks for acclimatization before use.

Treatment

Chemicals

Lead acetate and Pollen Bee propolis (500 mg tablets) were purchased from Aldrich, Germany and CC Pollen Co, U.S.A respectively. All other chemicals used were of analytical grade.

Forty male rats reared from this litter were divided into four groups of ten each: Group I**,** received 1 ml distilled water intraperitoneal (i.p.) for four weeks and served as a control group; group II**,** received propolis at a dose of 50 mg/kg/day daily orally (p.o.) for four weeks; group III, received lead acetate at a dose of 1 mg/kg/day, i.p. for four weeks (Patra et al., 2001). Lead acetate was dissolved in 1 ml sterile distilled water and group IV**,** received the above mentioned dose of Pb and propolis concomitantly for four weeks.

Sample collection

At the end of the study period, all rats were sacrificed by cervical dislocation under a mild dose of diethyl ether anesthesia, as per the procedure given by the Institutional Animal Ethics Committee. After sacrificed, the brain tissues were washed in cooled 0.9% saline and were excised and frozen immediately before further analysis. Brain tissues were used for the following analysis: Lipid peroxides (measured as malondialdehyde MDA), protein carbonyl contents (PCC), sulfhydryl proteins (P-SH) contents, vitamin E, vitamin C, mitochondrial enzyme (succinate dehydrogenase, SDH), mitochondrial respiratory chain enzymes (NADH-cytochrome C reductase and cytochrome C oxidase) and activity level of acetylcholinesterase (AChE).

Determination of MDA

To determine MDA in brain tissues according to the modified method described (Devi et al., 2006).

Determination of vitamin C and E

To determine vitamins C and E in the brain tissues, 10% homogenates in ice-cold 0.9% NaCl were prepared. Butylated hydroxyl toluene (BHT), 250 mmol/L in absolute ethanol, was then added. Next, the homogenates were centrifuged at 10000 g for 15 min at 4°C and the supernatant was kept frozen until assayed (Jurczuk et al., 2007).

Determination of acetyl cholinesterase activity

The total cholinesterase activity (AChE) in the brain was measured according to Ellman et al. (1961).

Determination of lipid peroxides (LPO)

Malondialdehyde (MDA), a reactive aldehyde that is a measure of lipid peroxidation, was determined according to the method of Uchiyama and Mihara (1979).

Determination of protein oxidation (measured as PCC)

Protein carbonyl content (PCC) was measured according to the procedure of Levine et al. (1990). The aliquots of water-soluble proteins containing 0.2 mg of total protein were treated with 10 mmol/L DNPH dissolved in 2 mol/L HCl, or with 2 mol/L HCl alone as blanks. Briefly, 10 mmol dinitrophenylhydrazine (DNPH) in 2.5 mol/L HCl was added to the tissue homogenate and incubated in the dark for 60 min at RT (stirred every 10 min). This was followed by vortex mixing, addition of 20% trichloroacetic acid (TCA) (w/v), and subsequent washing thrice with ethanol: Ethylacetate (1:1 v/v) mixture. Precipitated proteins were then redissolved in 6 mol/L guanidine HCl in 20 mmol/L phosphate buffer (pH 6.5). Insoluble substances were removed by centrifugation and absorbance of the supernatant was read at 370 nm. An extinction coefficient of 21.5 $nmolL^{-1}cm^{-1}$ was used to determine the protein carbonyl content and expressed as nmol/mg protein.

Determination of membrane-protein sulfhydryls (P-SH)

Membrane protein sulfhydryl concentration was measured spectrophotometrically using 5,5 dithio *bis*(2-nitrosobenzoic acid) (DTNB) as described by Ellman (1959). A 10% homogenate of the tissues was made in 5 mM sodium phosphate buffer, pH 8.0, centrifuged at 150 × g for 15 min and the supernatant was sonicated and centrifuged at 10,000 × g for 1 h. The pellet (membrane fraction) was solubilized in 10% sodium dodecylsulfate (SDS) in phosphate buffer. Aliquots of 200 µl of the solubilized membrane proteins, containing 100 µg protein, were mixed with 300 µl of 1 mmol/L DTNB, diluted to 3.3 ml with phosphate buffer, and incubated for 60 min at room temperature. Absorbance at 412 nm was recorded against the blank containing only DTNB.

Determination of vitamins C and E concentration

HPLC was applied to assay vitamin C (Ivanovic et al., 1999) and vitamin E (De Leenher et al., 1979) concentrations. To determine vitamin C concentration, 300 µl of meta-phosphoric acid (MPA) (100 g/L; stabilizes vitamin C and precipitates proteins) were added to an equal volume of the brain supernatant. Next, the samples

were centrifuged at 3500 g for 4 min at 4°C and 20 µl of the supernatant received were injected on the column for the HPLC analysis.

To assay vitamin E concentration, the samples were prepared as follows. Absolute ethanol, 250 µl, and 2.5 ml n-hexane were added to 250 µl of the brain supernatant and the samples were centrifuged at 3500 g for 10 min at 4°C. The upper hexane layer was transferred to a new tube, the hexane was evaporated under a stream of nitrogen, and the dry residue reconstituted in 250 µl absolute ethanol; 20 µl of such preparations were injected onto the HPLC column. All analyses were carried out on a TSP (Thermo Separation Products Inc.) HPLC system. The columns used were a reversed-phase Adsorbosil C18 column (Alltech, Baltimore, USA) (5 µm, 4.6 × 100 mm) for vitamin C and normal Ultrasphere Si column (Alltech, Baltimore, USA) (5 µm, 4.6 × 150 mm) for vitamin E. The analytical quality of the vitamin measurements was checked by repeated analyses and by the use of standard reference materials for ascorbic acid and α-tocopherol (Sigma Chemical Co, St Louis, MO, U.S.A.).

Isolation of mitochondria from brain

Mitochondria from the brain of the rat were isolated by differential centrifugation, according to the procedure of Clark and Nicklas (1970), as essentially described by Hillered and Emster (1983) with some modification. Briefly, the animals were killed and their brains were rapidly removed and put into an ice-cold medium containing 0.225 M mannitol, 0.075 M sucrose, 0.1 mM EGTA, 0.05% w/v bacterial proteinase (Nagarse) and 0.5% w/v fatty acid-free bovine serum albumin, pH 7.4. The tissue was cut into small pieces and washed with the cold isolation medium. Chopped tissue was transferred into medium (1:10, w/v) and homogenized by hand in a Potter-Elvehjem glass vessel fitted with a glass pestle. The homogenate was filtered through cheesecloth and the filtrate was centrifuged at 1000 g for 5 min at 4°C. The pellet was discarded and the supernatant fraction was centrifuged at 12,000 g for 15 min. The resulting mitochondrial pellet was gently resuspended in 3% Ficoll solution and layered over 6% Ficoll solution before centrifugation at 11,000 g for 30 min. The pellet was resuspended in 10 ml of a reaction medium consisting of 160 mM KCl, 10 mM potassium phosphate and 0.1 mM EGTA (pH 7.4) and centrifuged again for 15 min at 12,000 g to remove the bulk of the Ficoll, mannitol and sucrose. The final mitochondrial pellet was gently suspended in the reaction medium to a concentration of approximately 40 mg of mitochondrial protein per milliliter.

Measurement of NADH-cytochrome C reductase (Complex I) activity

Membrane bound mitochondrial activities were assayed spectrophotometrically in 100 mmol/L phosphate buffer (pH 7.4) at 30°C. For the determination of NADH-cytochrome C reductase activity, 2 mg of mitochondrial fragments were added with 0.2 mM NADH, 0.1 mM cytochrome C, and 1 mM KCN and absorption at 550 nm was followed (Yonetani, 1967). Enzyme activity was expressed in nanomoles cytochrome C reduced per minute per milligram of protein.

Measurement of succinate dehydrogenase (SDH) (Complex II) activity

Succinate dehydrogenase activity was determined spectrophotometrically by measuring the decrease in absorbance at 600 nm caused by the reduction of 2,6-dichlorophenol indophenol by succinate by the method of Vrbacky et al. (2007) with slight modications. To 1 ml phosphate buffer (0.1 mol/L, pH 7.6), 0.1 ml EDTA (1 mmol/L), 0.1 ml of BSA (1 mg/ml), 0.1 ml of sodium succinate (5 mM), 2.0 ml water, 0.1 ml potassium cyanide (1 mM) was added. To the above mixture, 0.2 ml of 1 in 5 diluted mitochondrial suspension was added. 0.1 ml of 2,6-dichlorophenol indophenols (0.1 mM) was added, mixed well and the absorbance was measured spectrophotometrically at 600 nm immediately against a reagent blank without the mitochondrial fraction and chromogen. The extinction co-efficient of 21 $mmolL^{-1}$ cm^{-1} was used for calculation. The specific activity of the enzyme was expressed in nanomoles of succinate oxidized/min/mg protein.

Measurement of cytochrome oxidase activity (Complex IV)

Cytochrome oxidase activity was determined with phosphate buffer (0.1 mol/L, pH 7.0) containing 0.1 mmol/L reduced cytochrome C, prepared by reduction with excess $NaBH_4$ and HCl, as the decrease in absorbance over 3 min at 550 nm (Yonetani, 1967).

Enzymatic activity was calculated in terms of the pseudo-first-order reaction constant for cytochrome C oxidation (k) per milligram of protein and expressed as the initial rate of cytochrome C oxidation in nanomoles cytochrome C per minute per milligram protein in the presence of 0.1 mmol/L cytochrome C.

Protein determination

The protein content was measured according to the method of Lowry et al. (1951).

Statistical analysis

Data were expressed as mean±SD. Comparison between different groups was analysed using two-way analysis of variance (ANOVA) with SPSS statistical software 11^{th} version (SPSS Inc., Chicago, IL). The level of significance was set at $p < 0.05$.

RESULTS

Effect of propolis on Pb-induced alterations in brain

The present results showed that, the propolis administration in healthy non-treated rats induced significant elevation in brain AChE activity as compared to the control group (Figure 1). Pb acetate administrated rats showed significant decrease in the AChE activity (21%) in the brain tissue as compared to the control group. Figure 1 also revealed that Pb and propolis (group IV) treated rats showed significantly decreased AChE activity (4.8%) in the brain tissue as compared to control group. On the other hand, group IV led to the significant increase the AChE activity (20%) in the brain tissue as compared to Pb-administered rats (Group III) (Figure 1). These results indicate that propolis has a partial recovery effect on brain AChE activity.

Propolis administration in healthy non-treated rats showed no significant change in brain MDA content as compared with control group (Table 1). While rats treated by Pb acetate (group III) showed significant increase in brain MDA content (83.54%) as compared with control

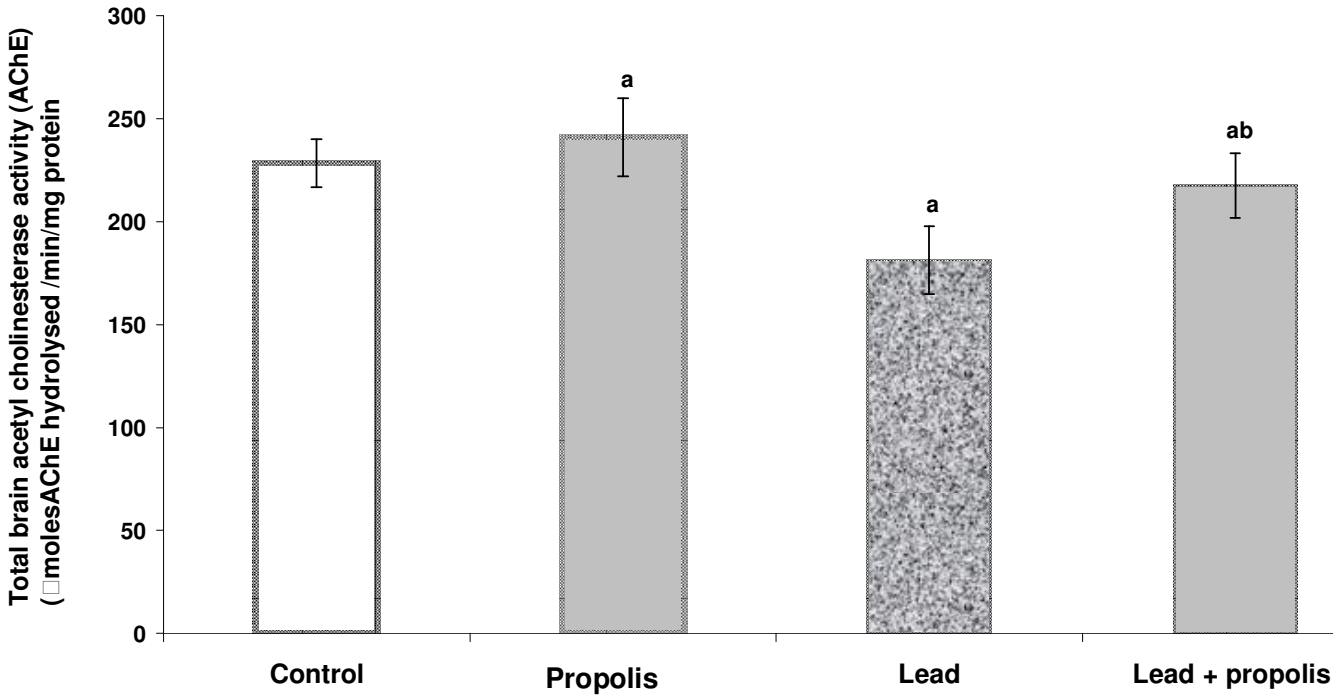

Figure 1. Effects of propolis on Pb-induced alteration sin total brain acetyl cholinesteraseactivity (AChE).
[a] Indicates significant difference compared to the control group, [b] Indicates significant difference compared with the Pb group (P <0.05). Data are mean ± SD of 10 rats.

Table 1. Effect of propolis on Pb-induced alterations in brain lipid peroxides (measured as MDA) and brain protein carbonyl content (PCC).

Group	MDA (nmol/g tissue)	PCC (nmol/mg protein)
Control (n=10)	96.00 ± 15.34	4.02 ± 0.91
Propolis (n=10)	84.20 ± 11.19	3.88 ± 1.01
Pb (n=10)	176.20 ± 12.10[a]	5.92 ± 0.45[a]
Pb + Propolis (n=10)	111.70 ± 10.86 [a b]	3.38 ± 0.66[b]

[a] Significantly different from control group, [b] Significantly different from Pb group (P <0.05), Data are mean ± SD of 10 rats.

group (Table 1). Table 1 shows the rats are administered with Pb and propolis (group IV) showed significant decrease in brain MDA content (36.60%) as compared with Pb group (III), while comparing this to control group, the MDA content was significantly (16.35%).increased in the brain tissue.

Table 1 shows propolis administration in healthy non-treated rats produced no significant change in brain PCC content as compared with control group. While rats treated by Pb (group III) showed significant increase in brain PCC content (47%) as compared with control group. On the other hand, rats administered Pb and propolis (group IV) showed significant decrease in brain PCC content (43%) as compared with groups-III; when this results is compared to control group, there was no significant change in brain PCC content (Table 1).

Table 2 illustrated that propolis administration in healthy non-treated rats showed no significant change in brain P-SH content as compared with control group, while rats treated by Pb (group-III) the P-SH content (16 %) significant decrease in brain tissue as compared to control group. Table 2 also shows that the rats administered by Pb and propolis (group-IV) resulted significant in increase in brain P-SH content (17%) as compared with III-group and when this data was compared to control group, it showed insignificant change in the P-SH content.

Propolis administration in healthy non-treated rats showed significant increase in brain vitamin C content (9.63%) as compared with control group .While rats treated with Pb (group-III) showed significant decrease in brain vitamin C content (17.72%) as compared with control group (Table 2). Further, of the rats administered with Pb and propolis (Group-IV) showed significant

Table 2. Effect of propolis on Pb-induced alterations in brain sulphydryl proteins (P-SH); brain vitamins C content and brain vitamin E content.

Group	P-SH (nmoles/g protein)	Vitamin C (µg/g tissue)	Vitamin E (µg/g tissue)
Control (n=10)	1.95 ± 0.32	300.20 ± 14.12	162.10 ± 11.51
Propolis (n=10)	2.09 ± 0.10	329.10 ± 15.27[a]	179.00 ± 9.59[a]
Pb (n=10)	1.63 ± 0.18[a]	247.00 ± 12.13[a]	131.20 ± 5.09[a]
Pb + Propolis (n=10)	1.91 ± 0.21[ab]	294.50 ± 16.39[b]	159.00 ± 9.31[b]

[a] Significantly different from control group, [b] Significantly different from Pb group ($P < 0.05$), Data are mean ± SD of 10 rats.

Table 3. Effect of propolis on Pb-induced alterations in brain mitochondrial NADH-cytochrome C reductase; succinate dehydrogenase (SDH) and cytochrome C oxidase activities.

Group	NADH-cytochrome C reductase (nmoles of NADH oxidised/min/mg protein)	SDH (nmoles of oxidised succinate/min/mg protein)	Cytochrome c oxidase (nmoles/min/mg protein)
Control (n=10)	156.10 ± 20.28	53.60 ± 3.81	390.10 ± 28.77
Propolis (n=10)	170.20 ± 10.35	56.50 ± 4.74	418.20 ± 30.39
Pb (n=10)	131.90 ± 5.47[a]	43.10 ± 3.45[a]	320.00 ± 19.41[a]
Pb + Propolis (n=10)	153.10 ± 7.37[b]	51.00 ± 2.71[b]	383.00 ± 19.90[b]

[a] Significantly different from control group, [b] Significantly different from Pb group ($P < 0.05$), Data are mean ± SD of 10 rats.

increase in brain vitamin C content (19.23%) as compared with III-group, when this data was compared to control group, it showed decrease in vitamin C.

Propolis administration in healthy non-treated rats showed significant increase in the vitamin E content (10.42%) as compared to control group. Further, the rats treated with Pb (group III) showed significant decrease in the vitamin E content (19.06%) as compared to control group. The rats administered Pb with propolis (group-IV) showed significant increase in the vitamin E content (21.19%) as compared to Group-III. While on this results comparing to control group showed insignificant in the vitamin E content (Table 2).

As shown in Table 3, propolis administration in healthy non-treated rats produced insignificant increase in brain mitochondrial NADH activity as compared with control group. While rats treated by Pb (group III) showed significant decrease in brain mitochondrial NADH activity (15%) as compared with control group. Also Table 3 revealed that treatment of rats administered lead by propolis (group IV) showed significant increase in brain mitochondrial NADH activity (16 %) as compared with Pb group (III), while on comparing to control group resulted in insignificant decrease in brain mitochondrial NADH activity as compared with control group.

Propolis administration in healthy non-treated rats showed that there was no significant variance in the mitochondrial succinate dehydrogenase (SDH) activity as compared to control group (Table 3). Rats administrated by Pb (Group III) showed significant decrease in brain mitochondrial SDH activity (20%) as compared to control group. The Pb administered rats (Group-III) treated with propolis (Group IV) showed significant increase in brain mitochondrial SDH activity (18%) as compared to Group-III, When this data was compared to control group, the result was insignificant.

Propolis administration in healthy non-treated rats showed insignificant increase in brain mitochondrial cytochrome C oxidase activity as compared with control group. Rats treated by Pb (Group III) showed significant decrease in brain mitochondrial cytochrome C oxidase activity (18%) as compared with control group. Pb treated rats were administrated with propolis (Group IV) resulted in significant increase in brain mitochondrial cytochrome C oxidase activity (20%) as compared to group-III; when this was compared to control group, it is insignificant.

DISCUSSION

The results of the present study revealed that co-treatment of lead-exposed rats with propolis protect the brain against Pb-induced inhibition in AChE activity.

The nervous system is the primary target for the lead-exposure and the developing brain appears to be especially vulnerable to Pb neurotoxicity (Basha et al., 2003). Lead has high affinity for free-SH groups in enzymes and proteins and its binding can alter their function (Bagchi et al., 1997). Inhibition of AChE activity in the present study is in agreement with the same

reports (Reddy et al., 2003, 2007). In addition, Pb competes with calcium (Ca^{2+}) and this may account for its disruption of cholinergic functioning and alterations in other transmitter systems (Silbergeld, 1977).

The present study showed that Pb-induced neurotoxicity is mainly by oxidative stress and mitochondrial dysfunction. In addition, It showed that Pb-induced neurotoxicity was characterized by various potentially detrimental changes such as inhibition of NADH-cytochrome C reductase, SDH and cytochrome C oxidase activities (mitochondrial function); and decreased P-SH, vitamin C and vitamin E; increased MDA and PCC levels (oxidative stress).

Propolis has been considered as a powerful ROS scavenger in rat and has neuroprotective effects through its antioxidant effects (Ozguner et al., 2005). González et al. (1999) found that calcium was the major element found in propolis dietetic products. Contrary to our results, recent study had shown that caffeic acid phenethyl ester (CAPE), one of the major components of propolis, increases gastric acid secretion stimulated by an acetylcholine agonist receptor likely through inhibition of AChE activity (Borrelli et al., 2005). These data could be explained by propolis has inhibitory and stimulatory effect on AChE activity according to its site.

Neurotoxicity associated with Pb exposure may be the result of a series of small perturbations in brain metabolism, and, in particular, of oxidative stress. Pb-stimulated lipid peroxidation resulted in the formation of aldehydic by-products, which in turn caused a decrease in reduced glutathione content (Gurer et al., 1998). In addition, the higher level of malondialdehyde in the brain of Pb-exposed rats indicates increased lipoperoxidation and potential neuronal membrane damage. In fact, the increased lipid peroxidation detected in the brain of lead-treated rats in the present study confirms previous results (Bennet et al., 2007).

In this study, propolis protects against Pb-induced lipid peroxidation and enhances reduced glutathione (GSH). Propolis has been reported to exhibit strong scavenging activity *in vitro* towards both the superoxide anion radical and the NO radical (Ichikawa et al., 2002). Dietary propolis significantly suppressed the lipoxygenase pathway and was shown to suppress the lipid hydroperoxide (LPO) (Mirzoeva and Calder, 1996) and enhances the levels of GSH (El-Khawaga et al., 2003). These findings of elevated GSH levels by propolis suggest GSH dependent detoxification of free radicals.

Failure to control reactive oxygen species accumulation leads to lipid peroxidation, enzyme inactivation, DNA damage and even cell death (Wu et al., 2003) and subsequently mitochondrial dysfunction. Reactive oxygen species can also attack directly at polypeptides of proteins and result in increasing carbonyl groups, the levels of which were described as metal-catalyzed oxidation of proteins (Stadtman, 1993) and were often taken as presumptive evidence of oxidative modification in protein (Levine et al., 1994). These data could explain suppression of mitochondrial enzyme activity (NADH-cytochrome C reductase, SDH and cytochrome C oxidase) and elevation of carbonyl protein in rats administered Pb in the present work.

Maier and Chan (2002) reported that, there is a link between oxidative damage and impaired mitochondrial function. Deveci (2006) demonstrated that, Pb induced ultrastructural changes in the form of vacularisation of cell cytoplasm, degeneration in mitochondria, electron-dense inclusion bodies were detected, and dilation were in the endopasmic reticulum.

Results of the present study are in agreement with Verma et al. (2006) where they observed a significant decline in the activities of the components of the electron transport chain, which are NADH-cytochrome C reductase, succinate dehydrogenase and cytochrome C oxidase in Pb-exposed rats. The results of the present study reflect the neurotoxic effects of Pb in terms of altered mitochondrial energy metabolism.

Propolis attenuated LPO and restored membrane integrity leading to maintenance of respiratory chain enzymes. Among the components of propolis, pinocembrin was one of flavonoids drawing much attention because of its benefits on human health due to anti-inflammatory, antioxidant and anti-allergic (Sala et al., 2003). Pinocembrin has protective effect on components of the mitochondria respiratory chain/oxidative phosphorylation system involving complex I activity, cytochrome oxidase expression, and origins of reactive oxygen species (Guang and Du, 2006). These data could explain the results of the present study.

In the present study, propolis induced reduction of carbonyl protein in rats administered Pb. Yong-Soo et al., (2004) reported that propolis significantly decrease carbonyl protein by its antioxidant effect.

Protein thiol groups (P-SH) may serve antioxidant function by several mechanisms; they may preemptively scavenge oxidants, which initiate peroxidation, thus sparing vitamin E and/or lipids from attack (Takenaka et al., 1991).

Hydroperoxides (P-OOHs) are major intermediates in radical-mediated protein oxidation reactions. In the current study, we have attributed the decrease in P-SH levels to the reaction of P- OOHs with -SH groups (Headlam et al., 2006). In this study, propolis increases the levels of P-SH within the brain and hence increases the antioxidant capacity of the cells resulting in a decrease in PCC enhanced by Pb-exposure. Patra et al. (2001) reported that Pb induced reduction in brain P-SH.

In the present study, lead-exposure decreases brain vitamin E and vitamin C contents. Similarly, recent study reported that Pb-exposure decreases vitamin E and vitamin C in rat liver and kidney homogenates (Jurczuk et al., 2007). In this study, reduced concentration of vitamin E and vitamin C in the brain after Pb-exposure may result from their exploitation during free radicals scavenging.

The antioxidative action of vitamin E and vitamin C involves first of all an inactivation of reactive oxygen species (ROS) such as superoxide anion radical, hydroxyl oxygen radical, hydrogen peroxide and singleton oxygen (Husain et al., 2001). Recent studies (Jurczuk et al., 2006, 2003) have revealed that Pb may induce lipid peroxidation by affecting the activity of antioxidative enzymes and GSH concentration. In addition, many studies provided evidence that vitamin C is a likely Pb chelator of potency similar to that of EDTA (Kulikowska-Karpinska and Kleszczewska, 2001). Thus, it could be assumed that the reduction in vitamin E and vitamin C concentrations in brain due to Pb exposure probably resulted from the vitamins interaction with the radicals produced during the Pb-induced lipid peroxidation and protein oxidation as well as vitamin C consumption in Pb chelation.

In this study, propolis increases the levels of both vitamin E and vitamin C within the brain and hence increases the antioxidant capacity of the cells resulting in a decrease in both lipid peroxidation and protein oxidation enhanced by Pb-exposure. Therapeutic activities of propolis depend mainly on the presence of flavonoids. The activities of enzymic and non-enzymic antioxidants were increased considerably on treatment with propolis (Padmavathi et al., 2006). Sun et al. (2000) reported that, propolis induced significant increase in plasma, kidney, stomach, small intestine, and large intestine vitamin C levels. They suggested that some components of propolis are absorbed to circulate in the blood and behave as a hydrophilic antioxidant that saves vitamin C.

Conclusion

The present study concludes that the AChE activity, oxidative stress and mitochondrial dysfunction could play an important role in Pb-induced neurotoxicity. Propolis attenuates Pb-induced neurotoxicity as it greatly improves AChE activity, oxidative stress and mitochondrial dysfunction.

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