Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(6):116-121
(http://derpharmachemica.com/archive.html)

Panax Ginseng Regulates Brain Monoamines in Lipopolysaccharide-Induced Experimental Brain Injury

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ABSTRACT

Administration of lipopolysaccharide (LPS) impaired antioxidant mechanisms, increased peroxidation and impaired mitochondrial redox activity causing brain inflammation as well as neuronal damage and impairment of brain monoamines. Panax ginseng (P.ginseng) is a well-known herbal medicine; the main active constituent of ginseng is ginsenosides or ginseng saponins that have neuroprotective activity. The aim of our experiment was to study the role of Panax ginseng root in maintaining brain monoamines levels through the protection against LPS - induced oxidative stress in rat model. In this experiment, we used forty rats and divided them into: control, ginseng, lipopolysaccharide and ginseng treated groups. malondialdehyde (MDA) and paraoxonase activity (PON-1) were estimated colorimetrically. Comet assay technique was used to determine the percent of DNA damage in addition to brain monoamines assessment by HPLC. The data showed that lipopolysaccharide significantly increased brain MDA, DNA damage percent and brain monoamines concomitant with a reduction in serum PON-1. Contrarily, ginseng supplementation improved these values in treated group. P. ginseng is a very important supplement that protects against brain injury and its benefit effect may be attributed to its high amounts of ginsenosides that have antioxidant, anti-inflammatory effects that augment impairment of brain monoamines.

Keywords: Oxidative stress, *ginseng*, neurotransmitters, HPLC, DNA damage.

INTRODUCTION

Lipopolysaccharide (LPS) is widely used in experimental animals to study the experimental inflammation and brain injury model. LPS was found to impair antioxidant mechanisms in addition to increasing peroxidation and disturbances of mitochondrial redox activity [1,2] causing brain inflammation [3] besides neuronal damage [4] and impairment of brain neurotransmitters[5].

Brain is a very sensitive organ to oxidative stress due to their high consumption of oxygen radicals and generation of large amounts of the reactive oxygen species (ROS) that are closely implicated in nervous system diseases including schizophrenia, Alzheimer's disease and Parkinson's disease (PD) [6].

It was reported that, generation of reactive oxygen species and oxidative stress are considered important pathogenetic mechanisms in the Parkinson's disease; [7] part of these free radicals are produced by autoxidation or enzymatically through the action of monoamine oxidase B during dopamine metabolism [8,9] besides their potential to analyze the neuroprotective compounds for their ability to act against these processes.

Ginsenosides (the active ingredients in *ginseng*) are known for their immune stimulant, anti-inflammatory, antioxidant and also neurotrophic properties. All these properties give *ginseng* their importance as a medicinal plant and their ability to protect neuronal cells against different neuronal diseases [10].

Various reports have shown the beneficial effects of *ginseng* or its ginsenoside components in the blockade of toxin uptake, [11] reductions in excitotoxicity [12, 13] and antioxidant effects, both through alterations in nitric oxide production as well as antioxidant enzyme activities required to eliminate free radicals, anti-inflammatory actions [14, 15] and altered expression of neurotrophic factors[16].

From this point of view we aimed to investigate the role of *Panax ginseng* root in maintaining brain monoamines levels in LPS-induced experimental brain injury.

MATERIALS AND METHODS

Chemicals

Lipopolysaccharide and neurotransmitters (dopamine, serotonin and norepinephrine) standards (HPLC grade) were obtained from Sigma Chemicals Company (USA).

Plant

Panax ginseng root was purchased from a local market and dissolved in ultrapure water**.**

Experimental animals

This experiment consists of four groups of animals, each group contains ten rats (male albino rats weighing 150 ± 10 gram) that were obtained from the animal house of the National Research Centre (NRC), Giza, Egypt. The animals were housed in individual suspended stainless steel cages at a controlled temperature, under 12-h light/12-h dark cycle, and allowed to acclimatize for a period of 10 days before the experiment; rats were allowed free access to food and water. The guidelines of the ethical care and animals' treatment followed the regulations of the ethical committee of National Research Centre.

Experimental design

Forty male albino rats were classified into four groups (10 rats in each group) as follows:

Group I: control group received a vehicle.
Group II: *ginseng* group received *ginseng* root powder dissolved in ultrapure water (22 mg /kg body weight / day) orally for ten days [17].

Group III: LPS group received a vehicle for ten days followed by intraperitoneal (i.p.) injection of lipopolysaccharide (LPS; 100 mg/kg body weight, once) [5].

Group IV: treated group received *ginseng* extract (22mg /kg body weight / day) orally for ten days followed by intraperitoneal (i.p.) injection of lipopolysaccharide (LPS; 100 mg/kg body weight, once).

After four hours of LPS injection, blood was withdrawn from the retro-orbital venous plexus of the eye using heparinized capillary tubes and collected in dry clean tubes for serum separation.

Brain was removed quickly, washed with cold saline solution (0.9% NaCl), homogenized and prepared for biochemical estimations.

Preparation of tissue homogenate

One gram of brain tissue was homogenized in five ml phosphate buffer , pH was adjusted at 7.4 then centrifuged at 4000 rpm at 4°C for 15 minutes using cooling centrifuge (Laborzentrifugen, 2K15, Sigma, Germany) ; the supernatant was then separated and used for estimation of biochemical parameters[18].

Biochemical analysis

Determination of brain lipid peroxidation

Malondialdehyde was determined by measuring thiobarbituric reactive species using the method described previously [19] in which the thiobarbituric acid reactive substances (TBARS) react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm.

Determination of serum paraoxonase activity

The aryl esterase activity of paraoxonase was measured spectrophotometrically in supernatants using phenyl acetate as a substrate. In this assay, aryl esterase/ paraoxonase catalyzes the cleavage of phenyl acetate, resulting in phenol formation. The rate of phenol formation is measured by monitoring the increase in absorbance at 270 nm at 25 º C. The working reagent consisted of 20 mM Tris/ HCl buffer, pH 8.0, containing 1 mM CaCl2 and 4 mM phenyl

acetate, as the substrate. Samples diluted in a ratio of 1:3 in buffer were added and the change in absorbance was recorded following a 20s lag time. Absorbance at 270 nm was taken every 15 s for 120 s using UV spectrophotometer [20].

Comet assay

Comet assay has been developed to detect cellular DNA damage according to Singh et al. [21] after modification of the previous method [22]. Lymphocytes were isolated and washed by phosphate buffered saline (PBS) at pH 7.4. Ten µl of the cells were suspended in 75 µl of 0.5% low melting agarose to pipette on microscopic slides with a layer of 1% agarose, spread using a cover slip and maintained on an ice-cold flat tray for 5 min. to solidify. After removal of the cover slip, the slides were immersed in cold lysis solution for 1 hour, followed by electrophoresis at 25 V, 300 mA, for 40 min. then, the slides were gently removed from the tank and washed three times with 0.4 M Trisma base at pH 7.5 for 10 min. Twenty µl from ethidium bromide (10 µg/ml) was added to each slide.

Visualization and analysis of comet slides

The slides were examined at 40× magnification using fluorescence microscope (Leica Microsystems, CMS GM b H, Wetzlar, Germany. Model DM 2500) and power Max. 160 W. equipped with an excitation filter of 549 nm and a barrier filter of 590 nm. Damaged cells were visualized by the "comet appearance", with a brightly fluorescent head and a tail to one side formed by the DNA containing strand breaks that were drawn away during electrophoresis. Samples were analyzed by counting the damaged cell out of 100 cells per slide to calculate the percent of damage.

Assessment of brain neurotransmitters by HPLC

Brain dopamine, norepinephrine, and serotonin were estimated according to the method described previously [18] using reversed phase HPLC column (5 µm, 25 x 0.46 cm) and quaternary pump (G131A model). The mobile phase was consisted of potassium phosphate buffer and methanol in a ratio of 97/3 (v/v) and a flow rate of 1 ml/min. UV detector was set at 270 nm; 20µl from different dilutions of standard also from each sample were injected onto HPLC. The concentration of dopamine, norepinephrine and serotonin were determined using standard curve.

Statistical analysis

The given data were expressed in the form of mean ± standard error (M± SE) and were analyzed using SPSS (Version16) by one-way ANOVA to assess differences between the mean of each group. When P value is less than or equal 0.05 it was considered a significant difference.

RESULTS

In the present study, intraperitoneal injection of lipopolysaccharide in a dose of (100 mg/kg body weight) significantly increased brain MDA in addition to DNA damage (P = 0.00) (figure 1) along with a reduction of serum PON-1 (*P = 0.00)* (table 1).

Table (1) : Oxidant / antioxidant parameters in brain tissue in different studied groups.

Groups / Parameters	Paraoxonase IU	MDA nmol/g tissue	%DNA damage
Control			
Mean ±SE	506.0 ± 27	274 ±13	8.5 ± .5
Ginseng			
Mean ±SE	514.0 ± 25	273.0 ±13	5.5 ± .5
P^a	0.021	N.S.	N.S.
LPS			
Mean ±SE	383.0 ±11	404.0 ± 13	39.5 ± 2.5
P^a	0.000	0.000	0.000
Treated			
Mean ±SE	452.0 ± 24	269.0 ± 19	29.0 ± 3
P^a	N.S.	N.S.	0.000
P^b	0.000	0.000	0.004

Significant value ≤ 0.05
P^a value compared to control group.
P^b value compared to LPS group.

Brain monoamines (dopamine, norepinephrine and serotonin) levels were significantly increased ($P \leq 0.00$) in LPS group compared to control, however, *Panax ginseng* supplementation significantly decreased these values ($P \leq 0.05$) compared to LPS group (table 2).

Table (2): Brain monoamines levels in different studied groups

Parameters / Groups	Norepinephrin (µg/g tissue)	Dopamine (µg/g tissue)	Serotonin (µg/g tissue)
Control Mean ±SE	2 ± .06	2.5 ± .3	2.2 ± .4
ginseng Mean ±SE	2.5 ± .08	3.2 ± .3	2.5 ± .2
P^a	N.S.	N.S.	N.S.
LPS Mean ±SE	6 ± .2	6 ± .8	4 ± .1
P^a	0.000	0.001	0.000
Treated Mean ±SE	2.4 ± .08	2.2 ± 1	2.8 ± .1
P^a	N.S.	N.S.	N.S.
P^b	0.000	0.001	0.002

Significant value ≤ 0.05
Pa value compared to control group.
Pb value compared to LPS group.

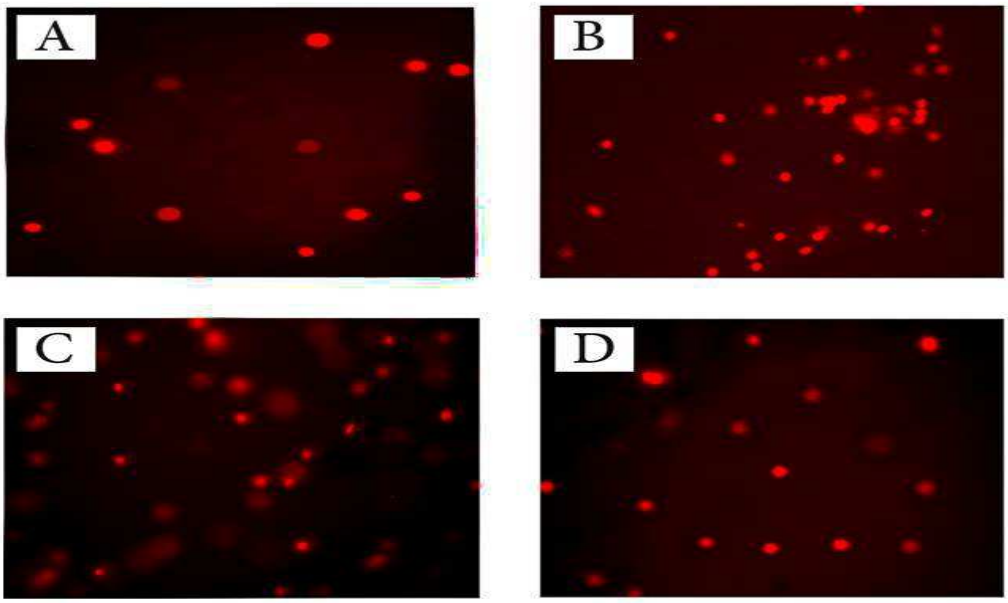

Figure1. Graphics of DNA damage appears normal DNA in A): control group (8.5%) and B): Panax ginseng group (5.5 %) compared to C) LPS group in which DNA damage elevated to 39.5% by lipopolysaccharide injection in a dose of (100 mg/kg body weight) and D) Panax ginseng treated group (29%).

DISCUSSION

ROS –induced oxidative stress cause tissue damage by different mechanisms including lipid peroxidation, protein oxidation, DNA damage and depletion of thiols. It is widely documented that the toxicity of aflatoxin in normal differentiated cells is a result of high levels ROS production [23]. Many scientists indicated that toxic agents are resulting in excessive lipid peroxidation [24], elevation of protein oxidation and DNA damage with a concomitant reduction of reduced glutathione (GSH) level [25].

Under normal physiological conditions, small amounts of lipid peroxidation occur in body tissues due to the high content of fatty acids in the cell membranes [26], these amounts will be elevated after cytotoxic agents such as lipopolysacharide.

In our experiment, intraperitoneal injection of lipopolysaccharide significantly increased MDA and DNA damage concomitant with a reduction of PON-1. These results are in agreement with our previous work [5] in which the

administration of LPS has been shown to impair antioxidant mechanisms, increase peroxidation and impair mitochondrial redox activity.

Paraoxonase enzyme that acts as an antioxidant agent and prevents the LDL oxidation is transported along with HDL in the plasma after synthesized in the liver [27].

In the current study, it was found that *P. ginseng* improved antioxidants' levels and decreased malondialdehyde levels in *ginseng* treated group when compared to LPS group. In agreement, other study found that ginseng extracts scavenge oxidative species [28]; also, it was indicated that *ginseng* extracts attenuate lipid peroxidation [29]. These results may be related to saponins which play a major role in antioxidant activities. In addition, ginsenoides have powerful antioxidant activities besides their radical scavenging activities by the stimulation of gene expression of antioxidant enzymes and improving their activities [30].

In addition to saponin (ginsenosides), *P. ginseng* contains many active compounds such as phenolic compounds, alkaloids, peptides, polyacetylenes and acidic polysaccharides [31]. However, ginsenosides are considered the main active compound in *P ginseng* in accordance to their pharmacological activities [32].

The elevation of brain monoamines in our study was augmented by ginseng administration treatment. We suggested this improvement in brain monoamines may be due to the antioxidant and anti-inflammatory effects of *Panax ginseng* that regulate releasing of brain monoamines. It was indicated that ginseng compounds may be used in treatment of memory deficits and improving central cholinergic function [33].

In the same line, it was observed that, ginsenosides enhanced norepinephrine and dopamine in cerebral cortex which explained the important effect of ginseng extract upon cognitive processing attention, integrated sensory-motor function and auditory reaction time in healthy subjects [34]. Indeed, it was shown that *P ginseng* total saponins modulates dopaminergic activity at both presynaptic and postsynaptic receptors, and blocks behavioral sensitization induced by psychostimulants such as morphine, cocaine, methamphetamines and nicotine. It was indicated that *P ginseng* regulated the releasing of serotonin [10]; they suggested that in rat brain, saponins enhanced biogenic amines. In addition, ginsenoside Rg2 interacted with nicotinic receptor subtypes directly besides regulation of GABA-ergic transmission in experimental animals.

Other suggestion was postulated by the previous study that indicated *P. ginseng* improved neuronal death and clinical behavior through regulation of inflammatory mediators (TNF-α, IL-1β, IL-6 and iNOS), microglial activation [35].

From these data, we concluded that *P. ginseng* is a very important supplement that protects against brain injury and its benefit effect may be attributed to its high amounts of ginsenosides that have antioxidant and anti-inflammatory effects that augment impairment of brain monoamines.

Acknowledgement
The authors are thankful to the National Research Centre (NRC) for supporting this work.

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