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Original Article

A study on the possible therapeutic role of Panax ginseng extract against a rat model of Parkinson's disease induced by intrastriatal rotenone injection

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Abstract: The neuroprotective effects of Panax ginseng were extensively studied. However, the therapeutic role of Panax ginseng in rat model of Parkinson's disease (PD) has not been studied enough. In the present study, rats were divided into three groups; control, rat model of PD induced by intrastriatal injection of rotenone and rat model of PD treated daily with Panax ginseng extract (100 mg/kg for 2 weeks). Forelimb wire hanging and the traction tests scored a significant decrease in PD model rats. In ginseng extract-treated group, these behavioral parameters changed to non significant values from the control rats. In the midbrain of rat model, a state of oxidative stress was observed as indicated from the significant increase in lipid peroxidation, nitric oxide and tumor necrosis factor-α and the decrease in reduced glutathione in comparison to control. This was accompanied by a significant decrease in dopamine and a significant increase in acetylcholinesterase activity. In the striatum, an increase in lipid peroxidation and a decrease in nitric oxide, dopamine content and acetylcholinesterase were recorded. Panax ginseng treatment improved all the midbrain and striatal changes induced by rotenone except nitric oxide. However, this improvement was partial since the measured parameters in ginseng-treated group were not significant from the rat model of PD except tumor necrosis factor-α. From the present findings, it could be concluded that Panax ginseng extract administration for 2 weeks showed a partial ameliorative effect against the rat model of PD induced by the intrastriatal injection of rotenone.

Keywords: Panax ginseng, Parkinson's disease, dopamine, oxidative stress, rat

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the progressive depletion of dopamine in the caudate/putamin (striatum) caused by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta [1]. Its most prominent clinical features include tremors at rest, bradykinesia, rigidity and postural instability [2].

In addition to the loss of dopaminergic neurons, there is a convergent evidence for early disturbances in cholinergic neurotransmission in PD [3], where the density of striatal cholinergic markers indicates an important role for cholinergic neurotransmission in striatal function [4]. These disturbances in cholinergic function have been suggested to mediate many of PD symptoms including dementia [5] and cognitive impairments [6]. The depletion of dopamine leads to an increased cholinergic activity in the striatum resulting in an imbalance between dopaminergic and cholinergic effects on the striatal control of the motor program [7].

Oxidative stress has been implicated in the pathophysiology of PD and this may result in neuronal damage and modulation of intracellular signaling pathways and consequently neuronal death occurs either by apoptosis or necrosis [8, 9]. Increased lipid peroxidation and reduced antioxidant enzyme activities have been observed in the brains of postmortem PD patients [10, 11] suggesting reduced capacity for detoxification in PD patients [12].

It has been reported that tumor necrosis factor-alpha (TNF-alpha), a potent pro-inflammatory cytokine, plays a promoting role in neuroinflammation-mediated progressive degeneration of dopaminergic neurons in PD [13, 14]. Furthermore, TNF-alpha-induced signaling pathway has been implicated in apoptotic cell death in PD [15]. It was significantly increased in the lumbar CSF and in the nigrostriatal regions of the brain in PD [15, 16].

It is clear that PD is a multifactorial disease characterized by self-perpetuating events including mitochondrial dysfunction, short-term and long-term oxidative and nitrosative stress, energy crisis, excitotoxicity, neuroinflammation and protein aggregation [17]. These events work in concert impinging on each other to promote cell death [18]. It is very likely that no single therapy will be sufficient and that multiple agents working through different mechanisms may offer the best hope for a future therapy.

Ginseng has been demonstrated to modulate several putative biochemical markers shown to be important to the initiation and progression of PD. Various reports have shown the beneficial effects of ginseng or its ginsenoide components in the blockade of toxin uptake [19], decrease in excitotoxicity [20], and antioxidant effects [21], both through changes in nitric oxide production as well as antioxidant mechanisms required to eliminate free radicals, anti-inflammatory actions [22] and altered neurotrophic factors expression [23]. This is because ginseng is a collection of different compounds called ginsenosides of which more than 60 have been identified each with unique actions [24].

Up to date, almost all studies concerning the effect of ginseng extract on PD and other neurodegenerative disorders used ginseng extract for neuroprotection not as a therapeutic agent. Therefore, our present study sheds light on the therapeutic effect of ginseng extract on the rat model of PD induced by a single intrastriatal injection of rotenone through measurement of dopamine, GSH, lipid peroxidation and TNF-alpha levels. In addition, assessment of AChE and GST activities and behavioral alterations were carried out.

Materials and methods

Animals

Male Wistar rats, obtained from the Animal House of the National Research Centre, Cairo, weighing between 230 and 250 g were housed under temperature- and light-controlled conditions with standard laboratory rodent chow and water provided ad libitum. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985).

Drugs and chemicals

Rotenone was purchased from Sigma Chemical Co. (St. Louis, MO, USA). It was dissolved in dimethyl sulfoxide (DMSO). Ginseng extract was obtained from Pharco Pharmaceuticals Co., Egypt. All other reagents were analytical grade reagents purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Surgical procedures

Rats were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.). After shaving the hair from the fronto-occipital area, antisepsis was performed with 2% iodine solution. A hole of 0.5 mm was made using orthodontic roof motor, and a number 2 drill to the right of the bregma until the dura matter was exposed. With the use of a Hamilton syringe fitted with a 30-gauge needle, the solution of rotenone (10 μg/3 μl/rat) was injected in the right striatum at AP 0.0, L-2.5, V 5.5 mm relative to bregma as described by Mulcahy et al. [25]. Using the previous coordinates, control rats were injected intrastriatally with DMSO (3 μl/rat). After the injection, burr hole was sealed with bone wax and antibiotic powder (neosporin) was sprayed at the incision.

Experimental design

After surgery, the animals were left for four weeks to establish the animal model of Parkinson's disease as described by Mulcahy et al. [25]. Then the animals of the Parkinson's disease model were divided into 2 groups. The first group received a daily oral administration

of saline and served as model animals. The animals of the second group received a daily oral administration of ginseng extract (100 mg/kg) for 2 weeks and served as treated animals. After surgery, the control animals were also left for four weeks and then received a daily oral administration of water for two weeks.

All animals were sacrificed 1 hour after the last administration. The brain of each rat was rapidly removed and divided into two halves. Each half was dissected into the striatum and midbrain, weighed and stored at -53°C until analyzed. The right half was homogenized in acidified butanol and used for the determination of dopamine. The left half was homogenized in 5% w/v 20 mM phosphate buffer, pH 7.6, centrifuged and used for the analysis of acetylcholinesterase (AChE) and glutathione-S-transferase activities and the levels of malondialdehyde (MDA) as a measure of lipid peroxidation, reduced glutathione (GSH), nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha).

Determination of dopamine

The quantitative determination of dopamine levels was carried out according to the method of Ciarlone [26] using a spectrofluorometer (Jasco FP-777, with a source of xenon arc lamp 150 watt, JASCO Ltd., Tokyo, Japan).

Determination of lipid peroxidation

Lipid peroxidation was determined in both the midbrain and striatum by measuring the thiobarbituric-acid-reactive substances according to the method of Ruiz-Larrea et al. [27]. Thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex which has a peak absorbance at 532 nm. The color was read in a Helios Alpha Thermospectronic (UVA 111615, England) spectrophotometer.

Determination of reduced glutathione

Reduced glutathione (GSH) was determined according to the Ellman's method [28]. This method is based on the reduction of Ellman's reagent by -SH groups of GSH to form 2-nitro-s-mercaptobenzoic acid which has an intense yellow color that can be measured spectrophotometrically at 412 nm.

Determination of nitric oxide level

Nitric oxide (NO) levels, measured as nitrite, were assayed using Griess reagent according to the method of Moshage et al. [29]. In this method, nitrite, a stable end product of the nitric oxide radical, is primarily used as an indicator for the production of nitric oxide. Nitrite is converted to a deep purple azo compound after the addition of Griess reagents. The purple/magenta color developed is read spectrophotometrically at 540 nm.

Determination of acetylcholinesterase activity

The procedure used for the analysis of acetylcholinesterase (AchE) activity was a modification of the method of Ellman et al. [30] as described by Gorun et al. [31]. The principle of the method is based on the measurement of the thiocholine produced as acetylthiocholine is hydrolyzed. The color was read immediately at 412 nm in a Helios Alpha Thermospectronic (UVA 111615, England) spectrophotometer.

Determination of glutathione-S-transferase activity

Glutathione-S-transferase was determined by measuring the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione which is accompanied by an increase in absorbance at 340 nm.

Determination of tumor necrosis factor alpha

Tumor necrosis factor-alpha (TNF-alpha) was measured using rat TNF-alpha Elisa kit which was obtained from Koma Biotech Inc. (Yeongdeungpo-Gu, Seoul 150-105, Korea). The developed color was read at 450 nm using a microtiter plate reader. The concentration was then calculated from a standard curve.

Behavioral tests

Forelimb hanging test: The wire hanging test was performed as described by Fan et al. [32] with modification. This maneuver tests neuromuscular and locomotor development [33]. Each rat was suspended grasping by its forelimb a wire (5 mm thick) 50 cm above a foam cushion and the time to drop was recorded. This procedure was repeated 3 times for each rat. Then the mean was calculated for each rat. A greater time was taken as an indicator of better strength or motor endurance.

Table 1. Effect of Panax ginseng extract on the levels of dopamine (μg/g), nitric oxide (μmol/g), TNF-alpha (μg/g), MDA (nmol/g) and GSH (mmol/g) and the activities of AChE (μmol SH/g/min) and GST (U/g) in the midbrain of rat model of Parkinson's disease

	Control	Rat Model of PD	D%	Rat Model of PD + Ginseng	D%	*P*-Value
Dopamine	$2.043^{a} \pm 0.449$ (7)	$1.377^{b} \pm 0.336$ (6)	-32.56	$1.541^{a,b} \pm 0.175$ (7)	-24.572	0.008
AChE	$2.220^{a} \pm 0.346$ (6)	$3.118^{b} \pm 0.567$ (6)	40.45	$4.793^{c} \pm 0.668$ (6)	115.901	0.000
Nitric Oxide	$0.389^{a} \pm 0.039$ (6)	$0.505^{b} \pm 0.083$ (6)	29.82	$0.543^{b} \pm 0.092$ (6)	39.59	0.008
TNF-alpha	$3.288^{a} \pm 0.361$ (6)	$4.241^{b} \pm 0.868$ (6)	28.98	$3.141^{a} \pm 0.458$ (6)	-4.47	0.013
MDA	$45.922^{a} \pm 5.226$ (6)	$56.922^{b} \pm 4.529$ (7)	23.95	$48.602^{a,b} \pm 7.560$ (6)	5.835	0.011
GSH	$4.171^{a} \pm 1.481$ (6)	$2.089^{b} \pm 0.571$ (6)	-49.92	$3.274^{a,b} \pm 0.563$ (6)	-21.51	0.018
GST	1.062 ± 0.139 (6)	1.103 ± 0.145 (7)	3.86	1.271 ± 0.215 (7)	19.68	ns

Values represent mean ± standard error with the number of animals between parentheses. D%: % difference with respect to control values [(Treated-Control)/Control] ×100. Statistically significant means (*P*-value < 0.05) are given in different letters. a: Non significant in comparison to control; b: Significant in comparison to control; c: Significant in comparison to control and rat model of PD.

Table 2. Effect of Panax ginseng extract on the levels of dopamine (μg/g), nitric oxide (μmol/g), TNF-alpha (pg/g), MDA (nmol/g) and GSH (mmol/g) and the activities of AChE (μmol SH/g/min) and GST (U/g) in the striatum of rat model of Parkinson's disease

	Control	Rat Model of PD	D%	Rat Model of PD + Ginseng	D%	*P*-Value
Dopamine	$4.572^{a} \pm 0.615$ (6)	$3.598^{b} \pm 0.510$ (6)	-21.30	$4.149^{a,b} \pm 0.740$ (6)	-9.25	0.05
AChE	$8.417^{a} \pm 1.376$ (7)	$3.096^{b} \pm 0.740$ (7)	-63.22	$4.642^{a,b} \pm 1.109$ (6)	-44.85	0.009
Nitric Oxide	$0.257^{a} \pm 0.090$ (6)	$0.149^{b} \pm 0.037$ (7)	-42.02	$0.161^{b} \pm 0.047$ (7)	-37.35	0.016
TNF-alpha	3.602 ± 1.161 (6)	3.310 ± 1.314 (6)	-8.11	2.836 ± 0.808 (7)	-21.26	ns
MDA	$39.551^{a} \pm 4.543$ (6)	$46.231^{b} \pm 2.456$ (6)	16.89	$45.551^{b} \pm 3.079$ (6)	15.170	0.01
GSH	3.044 ± 0.999 (6)	3.500 ± 0.991 (6)	14.980	3.789 ± 1.387 (7)	24.474	ns
GST	0.838 ± 0.120 (6)	0.688 ± 0.147 (7)	-17.899	0.864 ± 0.128 (7)	3.10	ns

Values represent mean ± standard error with the number of animals between parentheses. D%: % difference with respect to control values [(Treated-Control)/Control] ×100. Statistically significant means (*P*-value < 0.05) are given in different letters. a: Non significant in comparison to control; b: Significant in comparison to control; c: Significant in comparison to control and rat model of PD.

Traction test: The traction test was carried out as described by Dai et al. [34]. The rats were suspended by their front paws to a wire placed horizontally. Scoring was as follows; 3: the rat grasps the wire with two hind paws. 2: the rat grasps the wire with one hind paw. 1: the rat cannot grasp the wire with neither hind paws.

Statistical analysis

The data were expressed as means ± S.E.M. Data were analyzed by analysis of variance (ANOVA) followed by Tukey multiple range test when the F-test was significant ($P < 0.05$). All analyses were performed using the Statistical Package for Social Sciences (SPSS) software in a PC-compatible computer. The percentage difference (D%) was calculated as follows: D% = [Treated Value-Control Value/Control Value] ×100

Results

Neurochemical results

In the midbrain of rat model of PD (**Table 1**), a significant decrease in both dopamine and GSH was obtained as compared to rat model of PD. However, the levels of MDA, NO and TNF-alpha, and AChE activity increased significantly by 23.9%, 29.8%, 28.9% and 40.6% over the control values, respectively.

The daily treatment of PD model animals with ginseng extract for 2 weeks elevated the midbrain levels of dopamine and GSH to non significant changes from the control values and PD rat model values and attenuated the increase in lipid peroxidation to non significant changes in comparison to control and PD model values. Moreover, ginseng treatment restored the midbrain levels of TNF-alpha to control-like

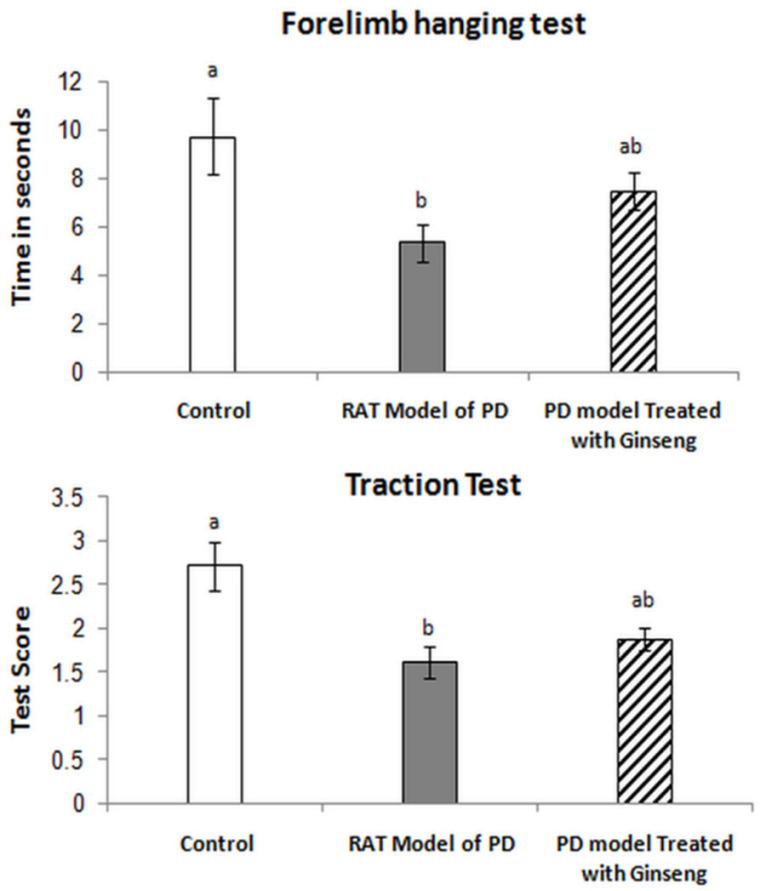

Figure 1. Effect of Panax ginseng extract on the forelimb hanging time (seconds) and traction test score of rat model of Parkinson's disease induced by intrastriatal injection of rotenone. Statistically significant means (*P*-value < 0.05) are given different letters.

values. However, the significantly elevated levels of NO and AChE activity recorded in the midbrain of PD rat model continued after treatment with ginseng extract.

In the striatum of rat model of PD (**Table 2**), a significant decrease in dopamine (-21.3%) and NO (-42.02%) levels and AChE activity (-63.22%) was observed. However, lipid peroxidation (16.9%) recorded a significant increase above the control value.

The daily oral treatment of rat model of PD with ginseng extract for 2 weeks elevated the dopamine content and AChE activity to non significant variation from control and PD model values. However, the recorded increase in lipid peroxidatin and decrease in NO in the striatum of rat model of PD persisted after treatment with the ginseng extract.

Behavioral results

Forelimb hanging test: As shown in **Figure 1** one way ANOVA revealed that there is a significant difference in the forelimb hanging time between control, rat model of PD and rat model of PD treated with ginseng extract. The hanging time decreased significantly in rat model of PD (-45.3%) below the control value. However, ginseng treatment increased the hanging time to be non significant from both the control and animal model.

Traction test: The score of the traction test was reduced significantly in the rat model of PD (-40.13%) compared to control. In addition, ginseng-treated rats also displayed a significant decrease in the score of traction test below the control value **Figure 1**.

Discussion

In the present study, the rat model of PD was induced by a unilateral intrastriatal injection of rotenone. This method can induce many of the neuropathlogical features of human PD as well as impairment in many motor functions related to the human condition [25]. Intrastriatal rotenone administration was used to overcome many of the drawbacks associated with systemic rotenone administration as organ toxicity [35], body weight loss [36] and increased mortality rates [37].

The present findings revealed that the unilateral intrastriatal rotenone injection induced a significant decrease in midbrain and striatal dopamine content. These neurochemical changes were accompanied by behavioral changes. Rotenone infusion decreased significantly the forelimb hanging time and traction test score below the control value. Thus, the present data support the occurrence of striatal and midbrain dopamine depletion and motor

dysfunction that represent the main hallmarks of PD.

It has been reported that rotenone induces highly selective dopaminergic lesion [38]. This could explain the significant decrease in the midbrain and striatal dopamine content observed in the present study.

The cell bodies of monoaminergic system are found in small nuclei in the substantia nigra, locus coeruleus or raphi nuclei and their axonal arborizations innervate large brain areas [39, 40]. The synaptic regulation of monoaminergic neurotransmission, which occurs mainly at the level of monoaminergic cell bodies, can only modify the function of the whole arborization such as dopaminergic neurons in substantia nigra and their axons in striatum [41]. Accordingly, it is obvious that the dopaminergic damage induced by rotenone resulted in a significant decrease in dopamine content in the midbrain, the site of dopaminergic cell bodies, and striatum that receives dopaminergic projections.

It is clear from the present findings that a state of oxidative stress was induced in the midbrain by intrastriatal rotenone injection. This was evident from the significant increase in lipid peroxidation and nitric oxide levels and the significant decrease in GSH; the nonenzymatic antioxidant. This was associated with a state of neuroinflammation that was mediated by the significant increase in TNF-alpha. Multiple studies showed that TNF-alpha is highly toxic to dopaminergic neurons both in vivo [42, 43] and in vitro [44]. In the striatum, rotenone induced a significant increase in striatal lipid peroxidation.

Thus, the states of oxidative and nitrosative stress together with the accompanying neuroinflammation could underlie the dopaminergic damage induced by rotenone in the midbrain and consequently the selective depletion of dopamine in the midbrain and striatum. Supporting this notion, it has been reported that dopaminergic neurons exert greater oxidative stress than other neurons due to the generation of hydrogen peroxide (H_2O_2) during monoamine oxidase-driven dopamine metabolism [45]. In addition, rotenone causes oxidative stress which exhausts the intrinsic antioxidant system resulting in cell death due to mitochondrial ATP depletion [45] and mitochondrial complex I inhibition [46]. Furthermore, rotenone activates the intrinsic apoptotic pathway which is characterized by mitochondrial membrane permeabilization, cytochrome-c release and apoptosome formation [47].

Extensive evidence indicates that NO has very important functions in the nervous system [48, 49]. Several in vivo [50, 51] and in vitro [52] studies revealed that NO evoked striatal dopamine release. Accordingly, the significant decrease in the striatal nitric oxide in the present study could inhibit the release of dopamine and this may exacerbate this pathological condition.

The remarkable abundance of acetylcholine and dopamine in the striatum suggests that they play critical roles in the functioning of the basal ganglia [53]. The loss of dopaminergic inhibition for increased cholinergic activity in the striatum causes an imbalance between dopaminergic and cholinergic modulation of the striatal output to the motor program. This favors an increased level of ACh which causes hyperactivity and due to continuous stimulation without inhibition, the characteristic symptoms of tremor, rigidity and muscle fatigue develop leading to postural instability [7].

Therefore, the recorded decrease in striatal AChE activity recorded in the present study could be due to its exhaustion to mitigate the increased striatal cholinergic activity where AChE is the enzyme responsible for termination of ACh action at its receptors by hydrolyzing it. This in turn could contribute to the reported increase in striatal ACh content and augment the imbalance between dopaminergic and cholinergic activity in the striatum.

Dopaminergic neurons in the substantia nigra pars compacta receive more dense projections from cholinergic pedunculopontine neurons (PPN) [54]. Activation of cholinergic neurons in the PPN increases dopamine release in the striatum and the nucleus accumbens through activation of acetylcholine receptors in the substantia nigra pars compacta and ventral tegmental area, respectively [55]. It has been suggested that PPN form a brainstem locomotor center which also degenerates in Parkinson's disease [56]. PPN dysfunction is associated with dopamine-resistant akinesia in Parkinson's disease [57].

The present increased midbrain AChE activity results in termination of the stimulatory effect of ACh on the dopaminergic neurons. This may be a feedback mechanism to alleviate the stress arising from the stimulatory role of ACh on still alive dopaminergic neurons as their number decreased due to the neuronal degeneration induced by rotenone.

Accordingly, the reported increase in midbrain AChE activity in the rotenone-treated rats could mediate the decreased cholinergic activity to substantia nigra pars compacta and consequently inhibits the still-alive dopaminergic neurons. This in turn may mediate and exaggerate the rotenone-induced decrease in dopamine content.

Supporting this explanation is the study of Chung et al. [58] who observed that the treatment with the AChE inhibitor donipezil for 6 weeks reduced the frequency of falls about 50% in frequently falling Parkinson's disease subjects.

In addition, impairment or degeneration of the basal forebrain cholinergic system may play a significant role in cognitive decline [59] and dementia [60]. Thus, the resulting decrease in cholinergic activity as a consequence of the increased midbrain AChE activity, in the present study, may underlie the reported dementia [61] and the impairment in cognitive functions [62] in Parkinson's disease subjects.

The present decrease in the nigrostriatal dopaminergic activity could mediate the observed significant decrease in the hanging time and the score of the traction test induced by rotenone.

In the midbrain, although daily ginseng treatment improved the decreased levels of dopamine and reduced glutathione and the increased level of lipid peroxidation induced by rotenone to non significant changes from the control, they still showed non significant changes from the rat model of PD. Moreover, ginseng restored the increased TNF-alpha to control-like values and increased the AChE activity above the control and PD model values. However, ginseng failed to restore the increase in nitric oxide induced by rotenone.

Similarly, in the striatum ginseng improved the decrease in dopamine level and AChE activity induced by rotenone to non significant changes from the control and rat model of PD. However, lipid peroxidation was still elevated after ginseng treatment.

The main active pharmacological compounds in Panax ginseng are ginsenosides which are derivatives of triterpenoid dammarane. More than 31 ginsenosides have been isolated from natural and processed Panax ginseng and novel ginsenosides continue to be reported [63].

The antioxidant activity of ginseng and its component ginsenosides has been reported in various tissues [64] including brain tissues [65] and were shown to have neuroprotective effects in both in vitro [21, 66] and in vivo [12, 67] models of PD. Ginseng extracts are composed of several ginsenosides that produce independent effects through multiple sites of action [68]. In a previous study, it has been observed that co-treatment of Rg1 with rotenone increases the survival of dopaminergic neurons, whereas pretreatment with Rg1 had no effect on rotenone-induced apoptosis [67]. In another study, it has been found that oral administration of Panax ginseng extract significantly and drastically blocked tyrosine hydroxylase (TH) cell loss in substantia nigra and reduced the appearance of locomotor dysfunction in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice [12]. In addition, Rg1 increased dopamine and its metabolites in the striatum and increased TH expression in substantia nigra of MPTP-treated rats [69].

It may be proposed that Panax ginseng extract could rescue the still-alive dopaminergic neurons against the neuronal degeneration induced by rotenone. This may explain the recorded improvement in midbrain and striatal dopamine content.

On the other hand, the ginsenoside Rg1 has been shown to suppress oxidative stress, block activation of JNK signaling [21, 70] and protect dopaminergic neurons [71]. Ginseng has also been found to eliminate free radicals by activating antioxidant enzyme activities [64]. In addition, pretreatment with Rg1 resulted in the reduction of dopamine-induced ROS and release of mitochondrial cytochrome C into the cytosol and inhibition of caspase-3 activation, inducible nitric oxide synthase protein level and

nitric oxide production in dopamine-induced PCl2 cells [21].

This may explain the observed improvement in GSH levels and reduction in lipid peroxidation in the midbrain after treatment of PD rat model with the ginseng extract. It is clear that the antioxidant activity of ginseng was more prominent in the midbrain than in the striatum.

Moreover, anti-inflammatory actions have been linked to the ginsenoside Rg3 and remediate upregulation of TNF-alpha, interleukin-1-beta and interleukin-6 mRNA along with iNOS and COX2 induction [72, 73]. In addition, it has been reported that ginsenoside Rd inhibits neuroinflammation of dopaminergic neurons induced by lipopolysaccharide treatment [74]. The anti-inflammatory action of ginseng is evident in the present PD model from its success in restoring TNF-alpha in the midbrain to nearly control values.

In the striatum of PD rat model treated with ginseng extract for 2 weeks, AChE activity was improved to non significant change from the control value. This elevation in AChE activity could suppress cholinergic activity and consequently the striatal dopamine/acetylcholine balance will be regained. The slight increment of striatal AChE activity after treatment of PD rat model with the ginseng extract represents a step toward the acquirement of the dopamine/acetylcholine balance in the striatum that has been disturbed in the PD model.

The elevated striatal AChE activity could be due to the restored inhibitory effect of dopamine on the striatal cholinergic interneurons that was absent in case of PD model. Dopamine may thus partially regain its role in the striatum in controlling the cholinergic activity.

However, in the midbrain ginseng treatment did not affect the increased activity of AChE. This could alleviate the stress arising from the stimulatory role of ACh on the midbrain dopaminergic neurons.

The present improvement in the striatal dopamine levels and AChE activity together with the improvement in midbrain dopamine, TNF-alpha, lipid peroxidation and GSH was accompanied by an improvement in the behavioral tests, the forelimbs hanging time and score of traction test. Thus, the present results support the conclusion of Cho [75] that Panax ginseng and its various ginsenosides may provide a potential means of slowing the progression of PD.

The present data indicate that although most of the neurochemical and behavioral parameters measured in the present study were not restored to control values, they showed an improvement approaching control values. The significant changes induced by rotenone compared to control values became non significant changes from the control and rat model of PD.

It could be deduced from the present preliminary findings that Panax ginseng extract administration for 2 weeks showed a partial treatment against the rat model of PD induced by the intrastriatal injection of rotenone. It is possible that a longer period of treatment may give more beneficial results; nonetheless, the present study throws light on the promising effect of ginseng as an adjuvant therapy against PD.

Disclosure of conflict of interest

None.

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