Original Article

Could Ginkgo Biloba Extract Has a Protective Effect on The Histological Changes Induced in The Substantia Nigra Pars Compacta of Parkinsonian Disease Model in Adult Male Albino Rat?

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ABSTRACT

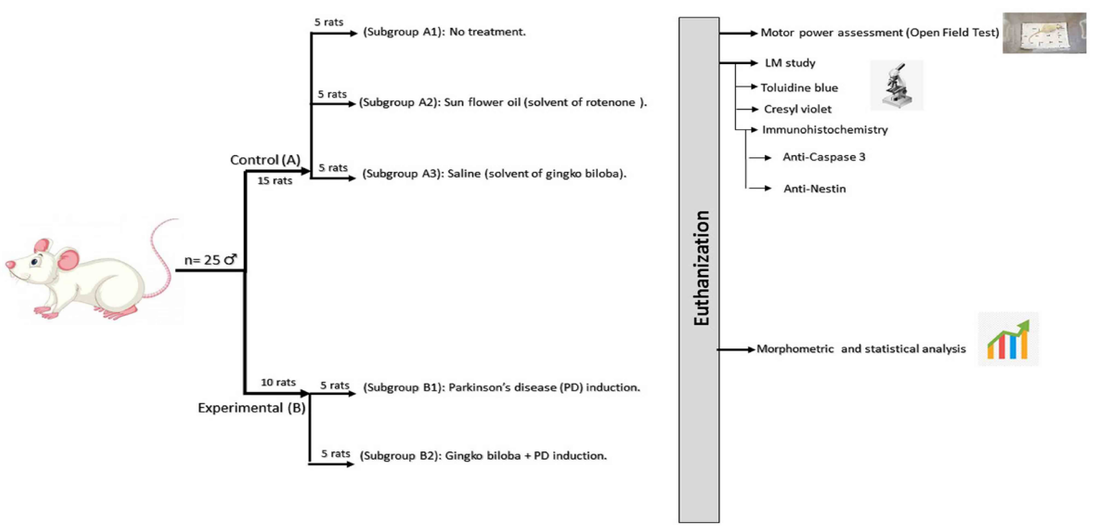
Introduction: Parkinson's disease (PD) is one of the most common neurodegenerative disorder. Rotenone was used to induce the neurochemical, neuropathological and behavioral features of Parkinson's disease. The neuroprotective & antioxidant effects of ginkgo biloba extract encourage using it.

Aim of the Work: Was to study the possible protective effect of ginkgo biloba extract on the histological structure of substantia nigra pars compacta (SNpc) in PD model of adult male albino rat induced by rotenone.

Material and Methods: The rats were divided into 2 main groups. Control groups (A) included 15 rats. Experimental groups (B) included 10 rats which were subdivided into 2 subgroups (5 rats each): Subgroup B1 (PD induced group) received rotenone at a dose of 1.5 mg/kg/day subcutaneously for 4 weeks and Subgroup B2 (ginkgo biloba + PD induced group) received ginkgo biloba extract at a dose of 100 mg /kg/day orally for 3 weeks then induction of PD as in subgroup B1.

Result: Subgroup B1 (PD induced group) revealed marked motor power impairment and degenerative changes of SNpc structure through which dopaminergic neurons appeared shrunken with small, dense nuclei and surrounded by wide halloes, Lewy bodies were seen by toluidine blue; apparent decreased number of Nissl bodies by cresyl violet and significantly increased mean optical density for caspase 3 protein, while significantly decreased mean optical density for nestin protein. On the other hand Subgroup B2 (ginkgo biloba + PD induced group) showed preservation of the normal motor power and histological structure of some dopaminergic neurons with few Lewy bodies were seen.

Conclusion: The present work showed that ginkgo biloba extract has a protective effect on the histological changes induced by rotenone in the substantia nigra pars compacta of parkinsonian disease model in adult male albino rat.



Graphical Abstract

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Key Words: Ginkgo biloba, immunohistochemistry, parkinson's disease, rotenone.

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INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative condition that occurs due to the gradual death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the midbrain[1]. Bradykinesia, aberrant postural reflexes, muscle stiffness and resting tremor are some of the clinical signs of Parkinson's disease. Additionally, cognitive abnormalities, depression and dementia are also common to occur in PD[2].

Rotenone is a naturally occurring cytotoxic chemical. It can gain access to all organs because of its high lipophilicity. It was discovered to cause the behavioral, neuropathological and neurochemical hallmarks of Parkinson's disease in rats[3].

Levodopa and other dopaminergic medicines are the mainstays of PD treatment. However, these medications only alleviate symptoms and do nothing to slow the deterioration of dopaminergic neurons. Besides the long-term use of these drugs causes them to lose some of their effectiveness and causes permanent side effects, so thinking about protection from this grievous disease is so important[4].

Neurodegeneration is a progressive loss of neurons from brain. Different diagnostic researches depends mainly on the estimation of different fluid and tissue biomarkers neglecting the histopathological evaluation of the nerve tissue. The latter, is a very important diagnostic tool that should be used to confirm the incidence of the neurodegenerative disease as well as to record its progression. Histopathological evaluation also has an important role to understand the nature of the neurodegenerative disease consequently, identification and resolving many diseases concerned with the public health. The neurodegenerative diseases are associated by proteinopathies especially Parkinson's disease. Through which misfolded proteins as well as stimulation of nerve cell injury was accompanied by increased cell expressions of caspases besides cytoskeletal protein changes especially nestin (a protein responsible for axonal growth and regeneration). These proteinopathies are now well identified using antibodies by immunohistochemistry methods; confirming the clinical picture of the disease[5].

A popular natural remedy is ginkgo biloba leaves. It is an alternative herbal medicine for neuroprotection. It can protect the nervous system by either a direct action on the neurons or by an indirect effect through the modulation of blood flow besides its antioxidant effects. It can remove oxygen free radicals, inhibit lipid peroxidation, and inflammation, allow for cell proliferation and protects against brain hypoxia. Consequently, improving the nerve cell energy metabolism besides its myelin protecting effects[6]. Furthermore, ginkgo biloba has a protective effect on the psychological and cognitive consequences of different neurodegenerative diseases[7]. As it could decrease the severity of motor symptoms, rigidity, and bradykinesia and intensity of rest tremors of PD. Through its modulating effects on proteinopathies and nerve cell injury by its antioxidant effects with the promotion of blood circulation. So, ginkgo biloba is considered to be very important in the scientific research to modulate different neurodegenerative disorders[8].

This study set out to answer the question, "Could ginkgo biloba extract has a protective effect on the histological changes that occur in the SNpc in adult male albino rat model of Parkinson's disease?".

MATERIAL AND METHODS

Chemicals

Rotenone was obtained as powder from Sigma-Aldrish Company for trading pharmaceutical chemicals and medical appliances in the United States.

Ginkgo biloba extract was obtained as oral solution (40mg/ml) from MEPACO (Arab Company for Pharmaceutical and Medicinal Plants) pharm, Cairo, Egypt.

Animals

The experiment was performed on 25 adult male Wistar albino rats that were aged 10-12 weeks and weighed 160-200 grams. The rats were housed in cages that were cleaned, well-ventilated, and maintained at a temperature of 26° C with 50% humidity and excellent lighting. They were also given water and a commercial laboratory meal that was comparable to what they would eat in the wild. They spent at least a week getting used to their new surroundings before the trial began. The animals were kept in the animal house of Histology Department, Faculty of Medicine, Tanta University. Approval code: 34612/4/21, from Tanta University's Faculty of Medicine's research ethical committee.

The rats were divided into two main groups:

Control groups (A): fifteen rats that were further divided into:

- Subgroup A1: It comprised five rats that were not given any treatments.
- Subgroup A2: included 5 rats that received sunflower oil 1 ml/kg/day (solvent for rotenone) subcutaneously for four weeks.
- Subgroup A3: included 5 rats that received daily 1 ml saline orally (solvent of ginkgo biloba extract) for three weeks.

Experimental groups (B): Ten rats that were further divided into:

Subgroup B1 (PD induced group): Five rats were given rotenone subcutaneously once daily for four weeks at a dosage of 1.5 mg/kg. Rotenone emulsion in sunflower oil with a final concentration of 1.5 mg/ml was used[9].

The calculated dose was obtained by dissolving 150 mg of rotenone in 100 ml sunflower oil. Each rat was subcutaneously injected 0.3 ml/200 gm body weight/rat of this preparation once daily for 4 weeks.

Subgroup B2 (ginkgo biloba + PD induced group): Five rats were given 100 mg/kg/day orally of a ginkgo biloba extract solution in saline for three weeks. After that, the rats were given rotenone at the same time and dose as the rats in the PD induced group[9,10].

Assessment of motor coordination and behavioral study of rats according to Tseng et al., 2020[11] by using Open Field Test

An open field test was used to monitor spontaneous locomotor activity using rectangular, open field apparatus measuring 60 × 40 × 50 cm. The floor of the apparatus was divided into 20 rectangular squares by pencil lines. Rats were placed individually in the center of the open-field and behavioral parameters were assessed for three minutes. Four motor parameters were quantified throughout this test: line crossings (the number of lines the rats crossed with all four paws) and rearing (the number of times the rats stood on their hind legs in the maze). The open field apparatus was cleaned after each session using 70% ethyl alcohol and permitted to dry between tests. The results for each group were recorded for statistical analysis.

Histological studies

All animals in each experimental group were sacrificed under the influence of pentobarbital anesthesia that was given intraperitoneally by dose 40 mg/kg body weight[12]. Perfusion fixation through the cerebrovascular system was done according to Brown *et al*., 2024[13].

Brains were dissected out (Figure 1A), cut transversely at the level of the midbrain (Figure 1B) to expose the substantia nigra (Figure 1C) under stereoscope (Carl Zeiss Suzhou Co., Ltd.3943028480, China).

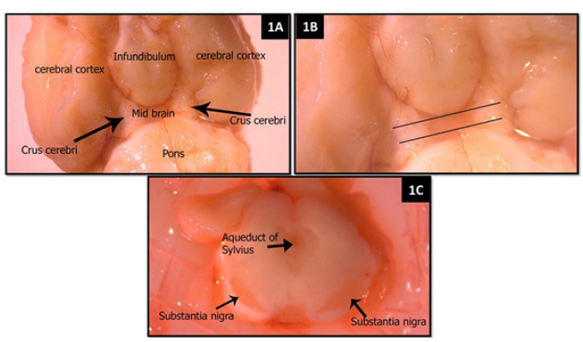


Fig. 1: (1A) showing brain and brain stem anterior view under stereoscope. (1B) showing site of dissection of midbrain under the stereoscope. (1C) showing transverse section in midbrain under stereoscope showing substantia nigra.

In the section of midbrain there is grey matter at the upper part surrounding aqueduct of sylvius. And in the lower part of section there is grey matter dorsal to each crus cerebri representing substantia nigra.

The substantia nigra is divided into a larger part consisting of a dorsal pars compacta (a cell rich zone comprising numerous densely packed neurons that contain melanin pigments) and a ventral pars reticularis (a cell poor zone whose neurons are enmeshed in the dense fiber network of the striatonigral fibers) and a smaller pars lateralis (comprising fibers and neurons dorsolateral and rostral in the most fibrous region of the substantia nigra).

Then midbrain specimens were processed for histological and immunohistochemical study. Finally by all necessary safety protocols and infection control measures; the slaughtered animals were carefully packaged and discharged.

Light microscopic study

Toluidine blue staining of semithin sections according to Woods and Stirling (2019)[14]. The specimens were fixed, dehydrated then infiltrated with epoxy resin and trimming of the blocks was done under dissecting microscope (Carl Zeiss Suzhou Co., China). The block surface was trimmed into a pyramid with the tissue in the apex having a small smooth trapezoid surface. Sections (1 µm thick) were cut by LEICA ultramicrotome (Leica Aktiengesellschaft Hernalser Hauptstr. 219 A-1171 Wein – Austria) and were mounted on glass slides, then stained.

Specimens for Cresyl violet and Immunohistochemical staining were dehydrated, cleared, impregnated in a pure soft paraffin and then embedded in hard paraffin. A rotary microtome (Leitz, 1512, Germany) was used to cut serial coronal slices at a thickness of 5 µm. The following procedures were used to stain the sections:

Cresyl fast violet according to Highley and Sullivan (2019)[15]

The sections were deparaffinized and hydrated to distilled water, then slide was incubated in Cresyl Violet Stain Solution (0.1%) for 2-5 minutes. Rinsed quickly in 1 change of distilled water, then Dehydrated quickly in absolute alcohol as alcohol may remove stain from tissue over time. Lastly, cleared in Xylene.

Immunohistochemical staining according to Hasic, (2022)[16]

After deparaffinization, sections were rehydrated and then incubated in a 10% hydrogen peroxide solution for ten to fifteen minutes. To retrieve the antigens, the sections were microwaved in a citrate buffer solution (PH 6) for 10–20 minutes. Parts were allowed to cool for twenty minutes in a room temperature environment. Using a buffer containing 0.05% sodium azide, the slides were washed twice. Primary antibodies were administered using monoclonal mouse anti-caspase 3 (1:50) cat. No. ARG57512 (to detect caspase protein which play essential role in programmed cell death so apoptotic cells can be detected) and anti-nestin (1:100) cat. No. ARG53358 (to detect nestin which is type VI intermediate filament responsible for radial growth of the axon) antibodies (Sigma Aldrich, Egypt). The slides were immersed in buffer four times. We used a secondary goat anti-mouse antibody that had been biotinylated (Nova Castra Laboratories Ltd, UK).

The slides were left to incubate at room temperature for ten minutes before being rinsed with buffer. A chromogen called diaminobenzidine (DAB) was used. A counter stain called Mayer's haematoxylin was employed. Human tonsil and kidney were used as positive controls for caspase 3 and nestin, respectively. While negative control slides were obtained by the same method with replacement of the monoclonal antibody by saline.

Morphometric study

Morphometric study was performed at Tanta University's Histology and Cell Biology Department, Faculty of Medicine, using an Olympus light microscope (Olympus, Japan). The software (Image J) program (National Institute of Health, Bethesda, Maryland, USA) was utilized. Using a 400x magnification, ten non-overlapping areas were taken from each slide to determine the mean color density of anti-caspase 3 and anti-nestin immunoreactivity.

Statistical analysis

Statistical Package for Social Sciences (version 11.5; SPSS IBM Incorp., New York, USA) was used to evaluate the data. One-way analysis of variance (ANOVA) test and a Tukey's test were applied to compare various subgroups. We presented all results as the mean plus or minus the standard deviation (SD). *P values* less than 0.05 were considered statistically significant, whereas *P values* less than 0.001 were considered extremely significant.

RESULTS

As regards the subgroups of the control group (subgroup A1,2&3); they showed the same results as regards motor power, toluidine blue, cresyl violet, anti-caspase as well as anti-nestin.

Motor power assessment by open field test: (Histograms 1,2, Tables 1,2)

Subgroup B1 (PD induced group) showed statistically highly significant decrease in the mean number of lines crossed ($p < 0.01$) (11 ± 2.345) and in the mean number of rearing ($p < 0.01$) (2.4 ± 1.140) when compared with control groups.

Concerning subgroup B2 (ginkgo biloba + PD group), there was significant decrease in the mean number of lines crossed ($p < 0.05$) (43.8 ± 3.768) and in the mean number of rearing ($p < 0.05$) (11.6 ± 1.140) when compared with control groups but highly significant increase ($p < 0.01$) when compared with subgroup B1 (PD induced group).

Toluidine blue

The histological structure of the control groups showed the normal SNpc as shown by light microscopic study of toluidine blue stained sections. Many dopaminergic neurons with rounded nuclei and large nucleoli surrounded by basophilic cytoplasm with the presence of intercellular neuropil appeared with several neuroglial cell types with basophilic nuclei (Figure 2A). In subgroup B1 (PD induced group) most dopaminergic neurons appeared shrunken and surrounded with wide halloes. Their nuclei appeared small and dense. Lewy bodies were present; in addition to vacuolated neuropil (Figure 2B). Subgroup B2 (ginkgo biloba + PD induced group) showed moderate preservation of their normal histological structure as certain dopaminergic neurons seem to be larger than others, with spherical nuclei, prominent nucleoli, and basophilic cytoplasm surrounding them. On the other hand, smaller dopaminergic cells with dense nuclei and broad halloes were also visible. Additional neuroglial cell types were seen, some of which had basophilic nuclei and few Lewy bodies were observed (Figure 2C).

Cresyl violet

Cresyl violet stained sections of control groups showed numerous purple blue stained Nissl bodies in the perikarya and dendrites of dopaminergic neurons (Figure 3A). While subgroup B1 (PD induced group) showed apparently decreased purple blue stained Nissl bodies in the shrunken perikarya of dopaminergic neurons (Figure 3B). Concerning subgroup B2 (ginkgo biloba + PD induced group), it showed apparently moderate amount of purple blue stained Nissl bodies in the perikarya of dopaminergic neurons (Figure 3C).

Anti-caspase 3

Positive control was from Human tonsil (Figure 4A). Negative control sections of SNpc showed no immunoreactivity for nestin protein (Figure 4B). In regards to the caspase 3 protein of control groups, it was observed that dopaminergic neurons exhibited very negligible cytoplasmic expression (Figure 4C). Dopaminergic neurons of subgroup B1 (PD induced group) exhibited significant cytoplasmic expression in response to caspase 3 protein (Figure 4D). Concerning caspase 3 protein of subgroup B2 (ginkgo biloba + PD induced group) showed apparently faint cytoplasmic expression in dopaminergic neurons (Figure 4E).

The mean optical density for caspase 3 protein in subgroup B1 (PD induced group) expressed as mean ± SD showed highly significant increase ($p < 0.01$) (64 ± 4.583) relative to control groups (5.2 ± 1.924). As regard subgroup B2 (ginkgo biloba + PD induced group), it showed highly significant decrease ($p < 0.01$) (11.6 ± 5.128) relative to subgroup B1 (PD induced group) (64 ± 4.583) and significant increase ($p < 0.05$) (11.6 ± 5.128) relative to control groups (5.2 ± 1.924) (Histogram 3, Table 3).

Anti-nestin

Positive control was from Human kidney (Figure 5A). Negative control sections of SNpc showed no immunoreactivity for nestin protein (Figure 5B). Dopaminergic neurons of control groups exhibited strong cytoplasmic production of nestin protein (Figure 5C). In subgroup B1 (PD induced group) there was very little cytoplasmic expression of the nestin protein

in the dopaminergic neurons (Figure 5D). Nestin protein showed apparently mild cytoplasmic expression in the dopaminergic neurons of subgroup B2 (ginkgo biloba + PD induced group) (Figure 5E).

The mean optical density for nestin protein in subgroup B1 (PD induced group) showed highly significant decrease ($p < 0.01$) (16.8 ± 1.924) relative to the control groups (70.4 ± 1.140). Concerning subgroup B2 (ginkgo biloba + PD induced group) showed highly significant increase ($p < 0.01$) (64.8 ± 3.782) relative to subgroup B1 (PD induced group) (16.8 ± 1.924) and non-significant change (< 0.05) (64.8 ± 3.782) relative to the control groups (70.4 ± 1.140). (Histogram 4, Table 4).

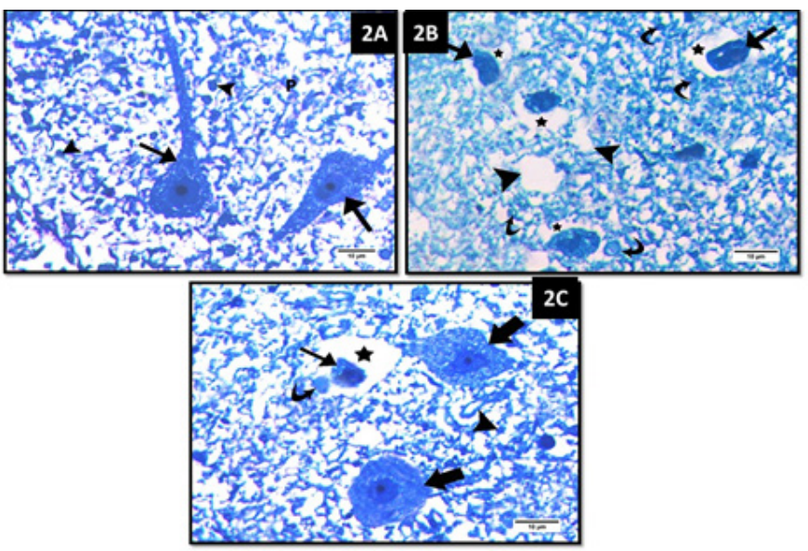


Fig. 2: Substantia nigra pars compacta stained by toluidine blue. (2A) control groups show numerous dopaminergic neurons with rounded nuclei, surrounded by localized basophilia of Nissil bodies in cytoplasm (arrow). The intercellular neuropil (P) consists of processes of nerve cells and processes of neuroglial cells. Different types of neuroglial cells appear smaller, darker and have dense nuclei (arrow head). (2B) Subgroup B1 (PD induced group) shows apparent shrunken dopaminergic cells with small, dense, irregular shaped nuclei (arrow) and surrounded with wide halloes (star). Notice: Lewy bodies (curved arrow) and the vacuolated neuropil (arrow head). (2C) Subgroup B2 (ginkgo biloba + PD induced group) shows some dopaminergic neurons with rounded nuclei, surrounded by localized basophilia in cytoplasm (thick arrow), apparent few dopaminergic cells are shrunken with small dense nuclei (thin arrow) and surrounded with wide halloes (star). Small nuclei of different types of neuroglia (arrow head) and few Lewy bodies are seen (curved arrow). (Toluidine blue X 1000, scale bar =10µm).

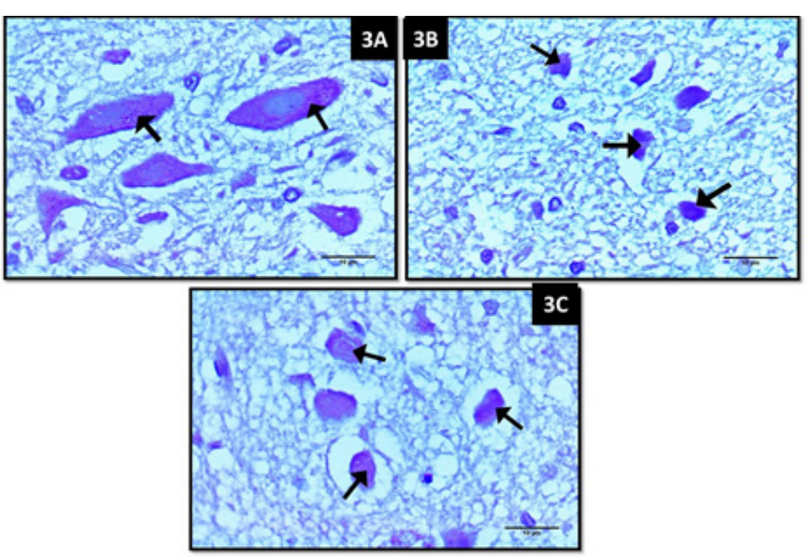


Fig. 3: Substantia nigra pars compacta stained with Cresyl violet: (3A) control groups show numerous purple blue stained Nissl bodies in the perikarya and dendrites of dopaminergic neurons (arrow). (3B) Subgroup B1 (PD induced group) reveals apparently decreased purple blue stained Nissl bodies in the shrunken perikarya of dopaminergic neurons (arrow). (3C) Subgroup B2 (ginkgo biloba + PD induced group) shows apparently moderate amount of purple blue stained Nissl bodies in the perikarya of dopaminergic neurons (arrow). (Cresyl violet X 1000, scale bar =10µm).

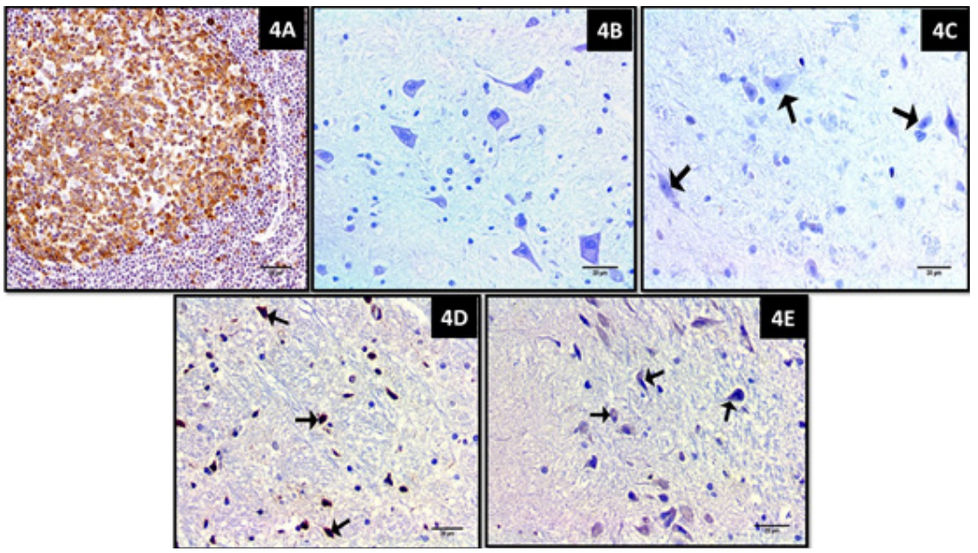


Fig. 4: Anti-caspase 3 immunohistochemical staining: (4A) Human tonsil tissue (Cell signaling technology) as positive control. (4B) Negative control sections of SNpc reveals no immunoreactivity for caspase 3 protein.(4C) control groups show nearly no apparent expression for caspase 3 protein in the cytoplasm of the dopaminergic neurons (arrow). (4D) Subgroup B1 (PD induced group) shows apparently strong cytoplasmic expression for caspase 3 protein antibody in the cytoplasm of the dopaminergic neurons (arrow). (4E) Subgroup B2 (ginkgo biloba + PD induced group) shows apparently faint cytoplasmic expression in the dopaminergic neurons (arrow). (Anti-caspase 3 immunostaining X 400, scale bar =20µm).

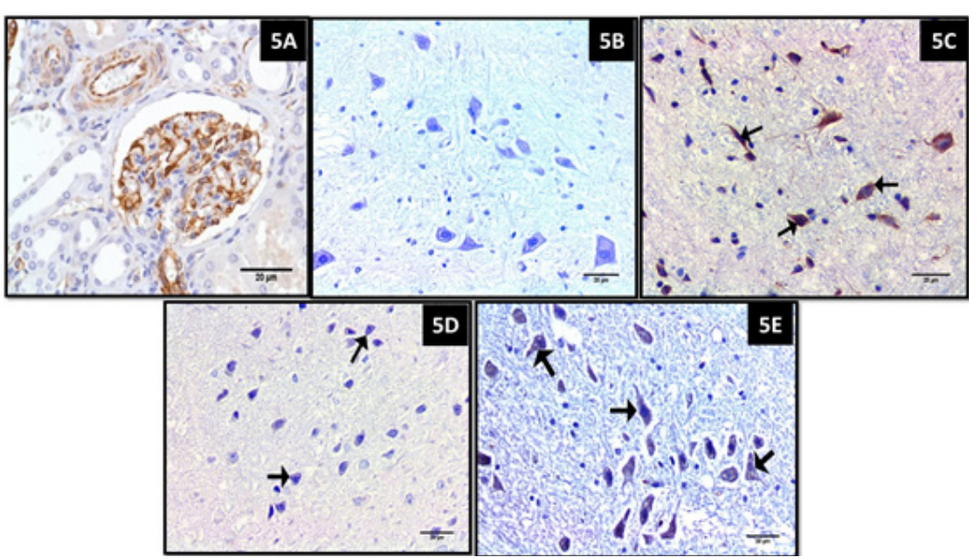


Fig.5: Anti-nestin immunohistochemical staining: (5A) Human kidney as positive control (arigo. Biolaboratories). (5B) Negative control sections of SNpc reveals no immunoreactivity for nestin protein. (5C) control groups reveal apparently very strong expression of nestin protein in the cytoplasm of the dopaminergic neurons (arrow). (5D) Subgroup B1 (PD induced group) shows weak expression for nestin protein in the cytoplasm of the dopaminergic neurons (arrow). (5E) Subgroup B2 (ginkgo biloba + PD induced group) shows apparently mild cytoplasmic expression of nestin protein in the dopaminergic neurons (arrow). (Anti-nestin immunostaining X 400, scale bar =20µm)

Table 1: The mean number of lines crossed in different studied groups expressed as mean ± SD

Group	Control groups	Subgroup B1 (PD induced group)	Subgroup B2 (ginkgo biloba + PD induced group)
The mean number of lines crossed	50.2 ± 3.834	11 ± 2.345*	43.8 ± 3.768** &***

*: $p < 0.01$ highly significant decrease relative to control groups; **: $p < 0.01$ highly significant increase relative to subgroup B1 (PD induced group); ***: $p < 0.05$ significant decrease relative to control groups.

Table 2: The mean number of rearing in different studied groups expressed as mean ± SD

Group	Control groups	Subgroup B1 (PD induced group)	Subgroup B2 (ginkgo biloba + PD induced group)
The mean number of rearing	14.8 ± 1.924	2.4 ± 1.140*	11.6 ± 1.140** &***

*: $p < 0.01$ highly significant decrease relative to control; **: $p < 0.01$ highly significant increase relative to subgroup B1 (PD induced group); ***: $p < 0.05$ significant decrease relative to control groups

Table 3: The mean optical density for caspase 3 protein in different studied groups expressed as mean ± SD

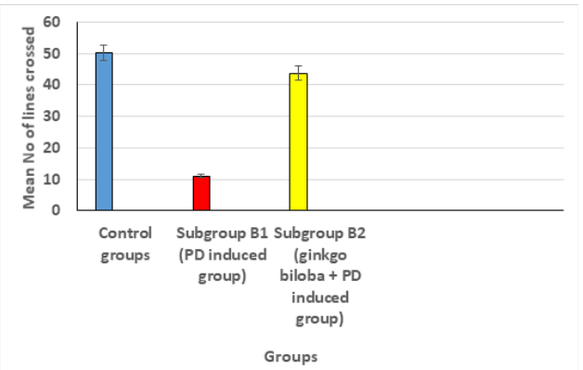
Group	Control groups	Subgroup B1 (PD induced group)	Subgroup B2 (ginkgo biloba + PD induced group)
Mean optical density for caspase 3 protein	5.2 ± 1.924	64 ± 4.583*	11.6 ± 5.128** &***

*: $p < 0.01$ highly significant increase relative to control groups; **: $p < 0.01$ highly significant decrease relative to subgroup B1 (PD induced group); ***: $p < 0.05$ significant increase relative to control groups

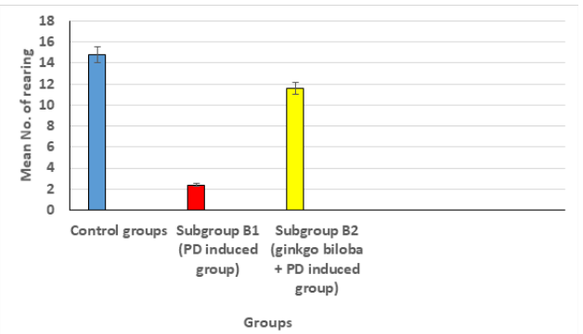
Table 4: The mean optical density for nestin protein in different studied groups expressed as mean ± SD

Group	Control groups	Subgroup B1 (PD induced group)	Subgroup B2 (ginkgo biloba + PD induced group)
Mean optical density for nestin protein	70.4 ± 1.140	16.8 ± 1.924*	64.8 ± 3.782** &***

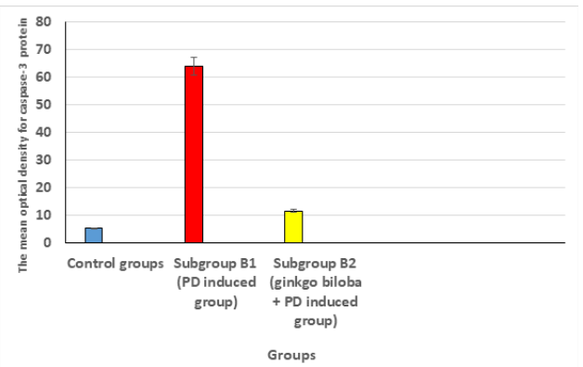
*: $p < 0.01$ highly significant increase relative to control groups; **: $p < 0.01$ highly significant decrease relative to subgroup B1 (PD induced group); ***: $p < 0.05$ significant increase relative to control groups



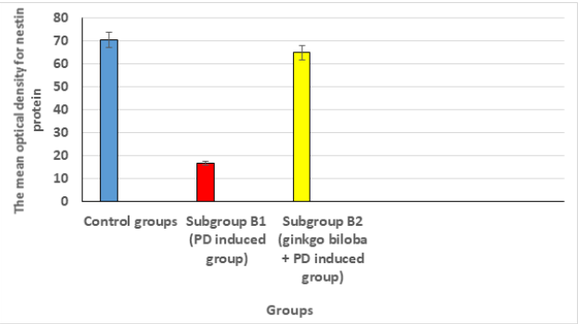
Histogram 1: The mean number of lines crossed in different studied groups expressed as mean ± SD *: $p < 0.01$ highly significant decrease relative to control groups; **: $p < 0.01$ highly significant increase relative to subgroup B1 (PD induced group); ***: $p < 0.05$ significant decrease relative to control groups.



Histogram 2: The mean number of rearing in different studied groups expressed as mean ± SD *: $p < 0.01$ highly significant decrease relative to control groups; **: $p < 0.01$ highly significant increase relative to subgroup B1 (PD induced group); ***: $p < 0.05$ significant decrease relative to control groups.



Histogram 3: The mean optical density for caspase 3 protein in different studied groups expressed as mean ± SD *: $p < 0.01$ highly significant increase relative to control groups; **: $p < 0.01$ highly significant decrease relative to Subgroup B1 (PD induced group); ***: $p < 0.05$ significant increase relative to control groups.



Histogram 4: The mean optical density for nestin protein in different studied groups expressed as mean ± SD *: $p < 0.01$ highly significant decrease relative to control groups; **: $p < 0.01$ highly significant increase relative to subgroup B1 (PD induced group); ***: $p < 0.05$ non-significant change relative to control groups.

DISCUSSION

Parkinson's disease (PD) is one of the most common neurodegenerative disorder[17]. Dopaminergic neurons of the substantia nigra pars compacta (SNpc) are destroyed, leading to a decrease in dopamine levels and the emergence of parkinsonian symptoms in rats. This is caused by highly selective dopaminergic degeneration and alpha-synuclein aggregation in dopaminergic neurons[18].

The present work was designed to study the protective effect of ginkgo biloba extract on the histological structure of SNpc in PD rat models. For such aim; motor power assessment, histological and immunohistochemical studies were done.

In order to assess the motor power of rats, the open field test was used in this study. This test showed the appearance of parkinsonian motor and behavioral manifestation in PD induced group. It exhibited impaired motor activity in the open field test as it revealed highly significant decrease in the mean number of lines crossed (The number of times the rats crossed one of the grid lines with all four paws) and the mean number of rearing (The number of times the rats stood on their hind legs in the maze) when compared to the control groups.

The parkinsonian motor manifestations occurred after rotenone exposure can be explained by the mechanism of action of rotenone as it exhibits a set of functional abnormalities in catecholaminergic neurons. These include a vesicular storage defect, decreased aldehyde dehydrogenase activity and subsequently increased dihydroxyphenylacetaladehyde (DOPAL). DOPAL is an important metabolite of dopamine which is detoxified mainly by aldehyde dehydrogenase. These defects lead to build up of DOPAL which play a major role in neurodegeneration and apoptosis[19].

The toluidine blue stained sections showed that the dopaminergic cells in the subgroup B1 (PD induced group) appeared smaller, with dense nuclei surrounded by wide halloes. Lewy bodies were also detected, in addition to vacuolated neuropil. Mbiydzenyuy *et al*., (2018)[20] confirmed these results as they noted shrunken dopaminergic neuron as a histological change of rotenone induced Parkinsonism.

Vacuolations that were observed in the neuropil of subgroup B1 (PD induced group) may be considered as a result of dopaminergic cells apoptosis leaving empty spaces. This finding was supported by Ragab and Mohamed (2017)[21], who discovered vacuolations in the neuropil in the cerebral cortex after administering tramadol.

Lewy bodies are the pathological hallmarks of PD. Which are produced after aggregation of a series of oligomeric, prefibrillar and fibrillar forms of alpha-synuclein[22,23]. The activity of the ubiquitin-proteasome system is impaired in the ventral midbrain when exposed to rotenone, leading to reduced protein clearance and an increase in cytoplasmic alpha-synuclein buildup[24].

In addition, the present study found that the cresyl violet staining of dopaminergic neurons in the perikarya of the subgroup B1 (PD induced group) showed a noticeable reduction in the number of Nissl bodies. This result might be due to endoplasmic reticulum (ER) stress which in turn causes neuronal degeneration and cell death[25]. Endoplasmic reticulum stress-mediated reactive oxygen species (ROS) generation and calcium deregulation causing activation of nuclear factor κB (NF-κB) which is a transcription factor that plays a crucial role in various biological processes including immune response, inflammation and cell survival[26].

Regarding the results of the immunohistochemistry, the use of anti-caspase 3 antibody to confirm cell death in the subgroup B1 (PD induced group) yielded a statistically significant increase in the mean optical density for caspase 3 protein compared to the control groups. This antibody detects caspase proteins, which are essential for programmed cell death and can be used to identify apoptotic cells. Rotenone exposure can activate caspase-9 and caspase-3 apoptosis, which in turn can upregulate apoptotic pathways and cause cytoplasmic release of cytochrome C, as shown in several investigations[27,28].

Neuronal development is facilitated by nestin, a type VI intermediate filament that is involved in both the construction and disassembly of these filaments. Compared to the control groups, the subgroup B1 (PD induced group) exhibited a highly significant drop in the mean nestin protein optical density. This may be due to neural cell death and accumulation of alpha-synuclein in these cells. This result is in agreement with Park *et al*., (2020)[29] who reported that nestin level decreases in dopaminergic neurons of PD rat model. Additionally, rotenone was proved to disturb the tubulin polymerization of microtubule selectively in dopaminergic neurons. This further impedes the transit of vesicles, which in turn increases oxidative stress[30].

The active ingredients in the extract of the popular herbal remedy ginkgo biloba include the terpene trilactones (ginkgolides and bilobalide) and flavonoids (such as

quercetin, kaempferol, and isorhamnetin)[31]. The positive benefits were determined to be largely influenced by these components[32,33]. The extract of ginkgo biloba possesses many beneficial effects including neuroprotection, anti-oxidant, in addition to its free-radical scavenging[6]. Therefore ginkgo biloba was used to examine its neuroprotective effect in PD.

Subgroup B2 (ginkgo biloba + PD induced group) showed moderate improvement of motor power assessment by open field test as it revealed significant decrease in the mean number of lines crossed and in the mean number of rearing when compared with the control groups but highly significant increase when compared with subgroup B1 (PD induced group).

The previous results can be explained as ginkgo biloba appears to act via antioxidant effect, free radical scavenging and stabilizing the mitochondrial membrane potential. So pre-treating rats with ginkgo biloba extract before induction of PD leads to a restoration of compromised cellular integrity to some extent[34].

In this study, Ginkgo biloba exerted a moderate protective role against the neurodegenerative effect of rotenone upon SNpc. Toluidine blue sections showed moderate preservation of the histological structure of SNpc. Some dopaminergic neurons appeared intact, while few dopaminergic cells appeared shrunken with small dense nuclei and surrounded with wide halloes. Intercellular neuropil showed different types of neuroglia with basophilic nuclei. Some Lewy bodies were also present. These findings were in agreement with EL-Ghazaly *et al*., 2015[10] who noted the neuroprotective effect of ginkgo biloba extract in a rat model of Parkinson's disease

As regard immunohistochemical staining, anti-caspase 3 antibody of subgroup B2 (ginkgo biloba + PD induced group) showed highly significant decrease in the mean optical density when compared with subgroup B1 (PD induced group). While anti-nestin antibody revealed highly significant increase when compared with subgroup B1 (PD induced group).

Since ginkgo biloba keeps the mitochondrial membrane intact, it stops cytochrome-c release and the apoptotic cascade and apoptosome from forming. This means that it has an anti-apoptotic effect[6]. It is worth mentioning that the kaempferol found in gingko biloba extract can enhance autophagic flow and hence protect against neurotoxicity caused by rotenone. This was reported by Siddique, (2021)[35]. In addition, the flavonoids found in ginkgo biloba, which are natural antioxidants, mitigate ubiquitin-proteasome system activity and inhibit apoptotic caspase activation, therefore reducing oxidative stress and preventing rotenone-mediated dopaminergic neurotoxicity in rats.[36].

CONCLUSION

From the previously mentioned data, it was concluded that ginkgo biloba extract can exert a protective role against rotenone –induced structural changes in the substantia nigra pars compacta of a model of parkinsonian disease in adult male albino rat.

LIMITATION

Limitation of this study was that the present work didn't show the effect of variable doses of ginkgo biloba extract. Also the study didn't compare the protective effect of other herbal medicine with ginkgo biloba.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

هل يمكن أن يكون لمستخلص الجنكو بيلوبا تأثير وقائي على التغيرات النسيجية المستحثة في المادة السوداء المضغوطة في نموذج مرض باركنسون فى ذكر الجرذ الأبيض البالغ؟

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خلفية البحث: يعتبر مرض باركنسون هو واحد من أهم التنكسات العصبية. الروتينون يسبب أعراض عصبية كيميائية وعصبية مرضية وسلوكية تشبه مرض باركنسون. التاثير الوقائى للأعصاب و المضاد للأكسدة لمستخلص الجنكو بيلوبا تشجع على استخدامه.

الهدف: هو دراسة تأثير الوقائى المحتمل لمستخلص الجنكوبيلوبا على التركيب الهستولوجى للمادة السوداء المضغوطة فى نموذج مرض باركنسون المستحث بواسطة االروتينون فى ذكر الجرذ الأبيض البالغ.

مواد و طرق البحث: تم تقسيم المجموعات إلى مجموعتين أساسيتين :المجموعات الضابطة (أ) شملت ١٥ جرذاً، المجموعات التجريبية (ب) شملت ١٠ جرذان التى إحتوت على مجموعتين فرعيتين (٥ جرذان لكل واحدة)؛ المجموعة الفرعية ب ١ (مجموعة مرض باركنسون المستحث): تم إعطاء الجرذان روتينون ١,٥ مجم/كجم/يوم تحت الجلد لمدة ٤ أسابيع، المجموعة الفرعية ب٢(مجموعة الجنكو بيلوبا + مرض باركنسون المستحث): تلقت مستخلص جنكو بيلوبا بجرعة ١٠٠مجم/كجم/يوم عن طريق الفم لمدة ٣ اسابيع ثم إحداث مرض باركنسون كما فى المجموعة الفرعية ب١ .

النتائج: أظهرت المجموعة الفرعية ب١ (مجموعة مرض باركنسون المستحث) إختلال كبير فى القوة الحركية و تغيرات فى تركيب المادة السوداء حيث إحتوت على العديد من الخلايا العصبية الدوباميئية المنكمشة و المحاطة بفراغات وذات أنوية صغيرة كثيفة و إحتوت على أجسام ليوي عن طريق صبغة التوليودين الزرقاء ؛ كما تبين إنخفاض عدد أجسام نيسل بواسطة صبغة الكريسيل البنفسجي؛ و أيضاً زيادة ذو دلالة إحصائية فى متوسط الكثافة البصرية لبروتين كاسباس ٣ بينما إنخفاض ذى دلالة إحصائية فى متوسط الكثافة البصرية لبروتين النيستين. من ناحية أخرى، أظهرت المجموعة الفرعية ب٢ (مجموعة الجنكو بيلوبا + مرض باركنسون المستحث) إحتفاظا للقوة الحركية و التركيب الهستولوجى لبعض الخلايا الدوباميئية مع القليل من اجسام لوى.

الاستنتاج: أظهر العمل الحالي أن مستخلص الجنكو بيلوبا له تأثير وقائي على التغيرات النسيجية لنموذج مرض باركنسون المستحث في المادة السوداء المضغوطة بواسطة الروتينون في ذكر الجرذ الأبيض البالغ.