RESEARCH ARTICLE

Effects of Irbesartan in induced Parkinson's Disease in Mice

Sarah J. Kamal*, Haitham M. Khadhim

Department of Pharmacology and Therapeutics, College of Medicine AL-Nahrain University, Iraq

ABSTRACT

Irbesartan (Irb) is an angiotensin receptor blockader (ARBs) and agonist of peroxisome proliferator-activated receptor (PPAR) γ, which owns inhibitory effects of inflammation, oxidation, and apoptosis, manipulation of the renin-angiotensin system results in dawdling nigrostriatal damage. The objective of the study is to explore the neuroprotective effect of Irbesartan on the dopaminergic neurons in the substantia nigra pars compacta (SNpc) in a 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine hydrochloride (MPTP) mouse model of Parkinson's disease (PD). Male mice were kept separately into 5 groups control (normal), model, classical treatment, oleic and Irbesartan group, the control group received N/S intraperitoneally (ip) (10 mL/kg) for 5 days, PD induced by MPTP IP (30 mg/kg) daily for 5 days for the all group except control, then all animal from day 6 to day 21 received a single oral dose of the following: control group and PD induced group (model or MPTP group) N/S (20 mL/kg), oleic acid 0.1 mL, Classical treatment (L-dopa/Carbidopa) [(250/25) mg/kg] and Irb (50 mg/kg) daily for 21 days. The behavioral changes of mice were assessed using a pole-climbing test. The levels of dopamine (DA), DA receptor type 2 (D2 receptors), caspase-3 and malondialdehyde (MDA) in the striatum were assayed with the help of the immunohistochemical method in substantia nigra. The results obtained from pole-climbing time in all test groups which performed each 5 days of the experiment (day 5, 10, 15, and 20) at day 5 revealed there were no significant change in time than model group ($p > 0.05$), at day 10,15 and 20 there were significant decrement in time for Irb group compared to model group ($p < 0.001$). The DA receptors and MDA decreased significantly in L-dopa, and Irb groups in compared to model group ($p < 0.001$), DA level were significantly increased in L-dopa and Irb in compared to model group ($p < 0.001$), while caspase-3 were significantly reduced in Irb in compared to L-dopa and model groups ($p < 0.001$). The result demonstrates the neuroprotective effect of Irb in PD's experimental model, suggesting Irb probably a candidate neuroprotective drug for human PD patients.

Keywords: Caspase-3, Dopamine, Dopamine receptor, Irbesartan, Malondialdehyde, MPTP, Neuroprotective effect, Parkinson's disease.

INTRODUCTION

Parkinson disease (PD) is a popular central nervous system (CNS) degenerative disease with decreased motor ability, muscle rigidity, tremor, bradykinesia, and inability to posture.[1] PD is relating the collapse of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) and resulting in the death of dopaminergic terminals in the striatum.[2] PD symptoms are due to the degeneration and loss of dopaminergic neurons in the SN and corpus striatum that followed by a sharp reduction in the neurotransmitter DA level in the corpus striatum and the formation of lewy bodies (LB) in cells.[3,4] Molecular studies in toxin-based cellular PD models propose that oxidative stress-mediated mitochondrial dysfunction, apoptosis, and microglia-mediated neuroinflammation own a major etiologic role underlying selective loss SNpc neurons.[5,6] The exact mechanisms of PD continue to be vague, former researches recommended that the neurochemical events related to the pathology of PD comprise elevated levels of free radicals, α-synuclein aggregation, raised levels of redox-active metals like iron and copper, raised lipid peroxidation, decreased glutathione levels,[7] mitochondrial dysfunction, neuroinflammation in addition to oxidative stress were included in this process, neurological collapse can be declined through chronic inflammation in the CNS.[8] Glial triggering-derived oxidative stress elevates the danger of emerging PD.[9] Neuroinflammation represents a main pathological PD mechanism and is a major target for PD treatment.[3,4] Apoptosis is caused from activated caspase proteolysis of different cellular constituents started by ROS production.[10] L-dopa was the initial medication utilized to substitute the DA.[11] L-dopa's difficulties are it owns a short half-life,[12] higher doses and extended-term intake of levodopa

Author for Correspondence: nadanahi9@gmail.com

are accompanied by side effects, such as motor instabilities and dyskinesia.[13]

To date, there is no treatment for PD though there are numerous conventional managements with a multiplicity of medicines and remedies, these cause alleviation of the symptoms and none appears to terminate the development of the disease.[14] Lately, researchers have started to learn about the mechanism of herbal effect and its monomer cure of PD; these mechanisms are largely included in saving dopaminergic neurons,[15] enhancing mitochondrial function, lowering neuritis, improving immune reaction, lowering excitotoxicity, anti-apoptosis, induction of autophagy, and preventing the heaped-up of odd proteins.[16] Natural compounds medicinal herbal extracts), are intended to act on multiple neural and biochemical objectives specifically.[17]

There is a local renin-angiotensin system (RAS) in the brain.[18] Angiotensin (Ang) II stimulates apoptosis of dopaminergic neurons via an AT1R-dependent manner in the SN[19] AII, via type 1 receptors (AT1R), is one of the most important known inducers of inflammation and oxidative stress, produces reactive oxygen species (ROS)[20] which is the most important intracellular source of ROS apart from mitochondria,[21] and plays a major role in the pathogenesis of several age-related degenerative diseases.[22] An antagonist of the AT1R, saves dopaminergic neurons.[23] The elevated glial inflammatory response and dopaminergic neuron susceptibility were found to be prevented by the AT1R antagonist.[24] Irbesartan (IRB), an ARB, is an identified agonist of peroxisome proliferator-activated receptor (PPAR) γ.[25] PPAR-γ functions a protecting action in anti-inflammatory reactions as a potent suppressor of the pro-inflammatory factor mediators in microglia and macrophages, neural inflammation via lowering the production of pro-inflammatory cytokines, chemokines, and adhesion molecules, prevention the stimulation of immune cells, and alleviate oxidative stress.[26]

AIM OF THE STUDY

To investigate the Anti-oxidant, anti-apoptotic, anti-inflammatory and neuroprotective effects effects of Irbesartan on experimentally induced Parkinson's disease and to investigate the effect of Irbesartan on biomarkers of Dopamine, Dopamine receptor, Caspase-3 and MDA on expermentally induced Parkinson's.

MATERIALS AND METHODS

Chemicals

Irbesartan powder (Sigma-Aldrich/ USA), Levodopa/carbidopa (Sigma-Aldrich/ USA), MPTP (Sigma-Aldrich/ USA) anti-caspase-3 antibody) Abcam / UK), anti-dopamine antibody)Abcam / UK), anti-dopamine receptor antibody) Abcam / UK), anti-Malondialdehyde antibody (MyBiosource/ USA), caspase anti-body kit (Abcam/ UK), oleic oil (Thomas Baker/ India). All other chemicals are of the highest purity obtained from reputed sources. Animal feeding gavage curved 20G×1″ (2 mm tip diameter) Reusable (USA).

Drugs Material

Preparation of Irbesartan Stock Solution

(1000 mg) of pure Irbesartan powder weighted by using electronic balance in a beaker and dissolved in (123 mL) of oleic acid[27] to get (1.63 mg/0.2 mL) (50mg/kg)[28,29] given orally in single dose via gavage for 21 days.[30]

Preparation of L-dopa/Carbidopa Stock Solution

(2000 mg/200 mg) of L-dopa/Carbidopa weighted and dissolved in (100 mL) of 0.1 N 10% HCL to get [(8 mg/0.8 mg)/ 0.4 mL] [(250mg/25mg)/kg][31] and the preparation of stock solution is as follow (11): 0.1 N 10% HCL is prepared by measuring (14.3 mL) of HCL by graduated cylinder then volume completed with distilled water up to (50 mL), shaking 20 times to get homogenous solution. L-dopa/Carbidopa powder placed in 250 mL beaker, and (100 mL) of 10% HCL is added. The beaker was placed on magnetic stirrer with the presence of magnetic bar and stirred for 1 hour. Stored in cold dark place. Given orally in single dose via gavage for 21 days.[30]

Induction of Parkinson's Disease

Induction of PD by single intraperitoneal (IP) injection of MPTP for 5 consecutive days. The powder weighted (50 mg) and dissolved in (5 mL) water for injection to get (1 mg/0.2 mL) (30mg/kg).[32]

Animal

Forty apparently healthy albino male mice 2-3 months age, weight about 30-40g, were included in the study and were randomly divided into 5 groups (8 mice each). Mice were purchased from the serum and vaccine institution-Al Amiriya, and from the animal house of the College of Pharmacy, Baghdad University and maintained under conditions of controlled temperature and humidity and light/dark cycle in the animal house of the College of Pharmacy, University of Baghdad. The animals in each group were kept in a separated plastic cage and fed standard laboratory pellets and tap water *ad libitum* for a week before the commencement of the experiment.

The study protocol was approved by the Institutional Review Board at the College of Medicine, Al-Nahrain University.

Study Design

The animals were divided into 5 groups of 8 animals for each, group 1 (control group) received N/S intraperitoneally (ip) (10 mL/kg) for 5 days, PD induced by MPTP IP (30 mg/kg) daily for 5 days for the all group except control, then all animal from day 6 to day 21 received single oral dose of the following: group 1 (normal or control group) and group 2 PD induced group (model or MPTP group) N/S (20 ml/kg), group 3 (oleic acid) 0.1 mL,[33] group 4 classical treatment (L-dopa/ Carbidopa) [(250/25) mg/kg] and group 5 (Irb) (50 mg/kg) daily for 21 days. The behavioral changes of mice was assessed using a pole-climbing test.The levels of Dopamine (DA), DA receptor type 2 (D2 receptors), caspase-3, and malondialdehyde (MDA) in the striatum were assayed with the help of the immunohistochemical method in substantia nigra.

Parameters of Study

Behavioral changes, DA receptors (D2-receptor), DA, caspase-3 and MDA level in nigrostriatal tissue.

Behavioral Assessment

PD induced mice went through the pole-climbing tests to analyze the variance in behavior.[34] A plastic foam ball (2 cm Diameter) was kept on a vertically fixed 50 cm long wooden pole (with 1 cm diameter) on the floor. To prevent skid possibility, 2 layers of gauze were put on the surface of pole. Further to that, the mice was subjected to the top of a pole and let to climb down without any other effect.

The testing was stopped as soon the double forelimb of mice came in contact with the pole's bottom, and pole-climbing time was taken. It continued further on day 5, day 10, day 15, and day 20 while administering the treatments.

Immunohistochemical Analyses

On day 22 the mice were anesthetized and scarified by cervical dislocation. The brains of the mice were removed and fixed in the paraformaldehyde overnight; further to this, brains were dehydrated in ascending alcohol series, cleared in xylene, infiltrated with and then embedded into paraffin. The 5 μm coronal sections were taken and stained for immunohistochemistry (IHC), sections deparaffinization, dewaxing of paraffin inserted section was put inside hot air broiler at 65°C for 30 minutes then by inundating the slides in xylene for 5 minutes and further in new xylene for 5 minutes. The sections were rehydrated in descending alcohols by methods of using absolute ethanol for 5 minutes, 95% of ethanol for 5 minutes, 90% of ethanol for 5 minutes, 70% of ethanol for 5 minutes, 50% of ethanol for 5 minutes and at last distilled water for 5 minutes. After this, the sections were immersed in 10% H2O2/methanol to reduce endogenous peroxidase activity for 10 minutes. Then, the sections were washed in Tris buffer [pH 7.4]. Further to washing, the sections were incubated in BSA for 10 minutes. After 1-hour incubation of the primary antibody sections, they have washed again in Tris wash buffered (pH 7.4) and subjected to one-hour incubation in the secondary antibody. The sections were washed in Tris (pH 7.4) and to visualize the bound antibody, they were subjected to react with DAB for 10 minutes, washed in Tris (pH 7.4), and Counterstaining was done with Myers' Hematoxylin for 2min, washing with tap water was then followed. Then section was mounted` with super mount and coverslip added and examined under a light microscope.[35]

Evaluation of the Immunostaining

Evaluation of IHC results for performed by light microscope (Genex 20, America) at 40X objective lens with a total power of magnification 400X. Slides were examined with a microscope to determine the level of neurodegeneration in SNpc.

Statistical Analysis

Data analyzed and presented using two statistical software programs: the statistical package for social science (SPSS version 23) and Microsoft Office Excel 2016. All results of markers were presented as median, mean and standard deviation. Two groups were compared using Mann Whitney U test and Kruskal Wallis test when compared more than two groups. Regarding behavioral changes, data were presented as mean ± SD, and comparison of mean values between two groups was done using unpaired t-test, while analysis of variance (ANOVA) was done when compare means of several groups with post hoc Tukey test. $p \leq 0.05$ was considered significant and highly significant when $p < 0.001$.[36]

RESULTS

Behavioral Changes

A significant difference between induced group and control group in assessing behavioral change at all pole test days, on the other hand no significant difference between induced group and other groups at day 5, also no significant variance for the induced group in comparison with an oleic acid group at all days of the test (5, 10, 15 and 20) ($p > 0.05$), while there were significant reduction in time between induced group and classical Tx and Irb group at day 10, 15 and 20 ($p < 0.001$) seen in Table 1 and Figure 1.
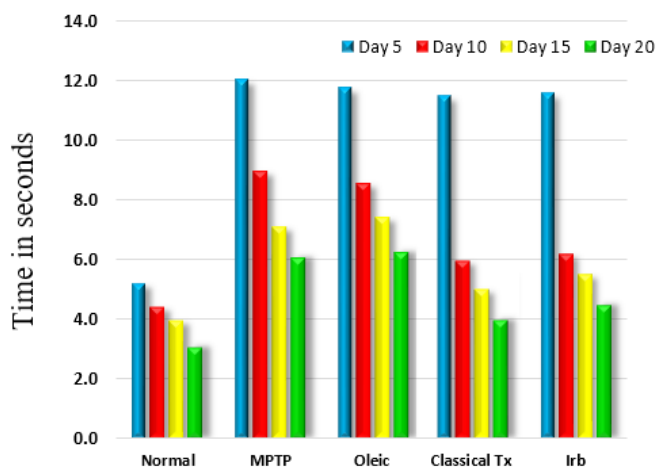

Figure 1: Comparison of days in all study groups Normal (Control) group (1), MPTP (induction) group (2), Oleic acid group (3), Classical treatment group (4) and Irbesartan group (5)

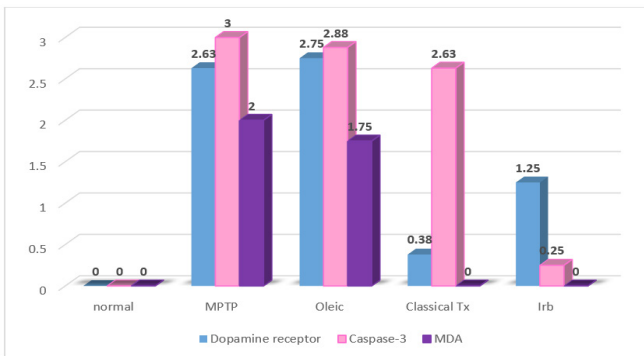**Table 1:** Comparison between all drugs groups

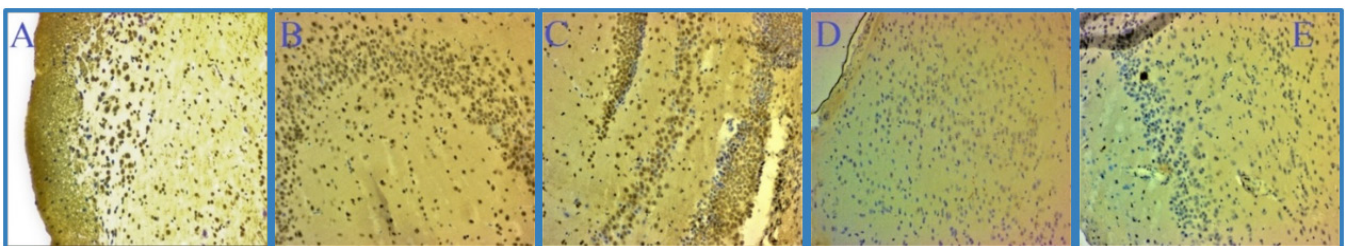| Days	Normal N=8 time in sec	MPTP N=8 time in sec	Oleic N=8 time in sec	Classical Tx N=8 time in sec	Irb N=8 time in sec	p-value*
Day 5	5.2 + 0.32	12.04 + 0.94	11.75 + 1.38	11.51 + 1.32	11.58 + 1.37	0.913
Day 10	4.44 + 0.39	9.0 + 1.27	8.6 + 1.13	6.0 + 0.59	6.2 + 0.38	<0.001
Day 15	3.96 + 0.16	7.13 + 0.75	7.43 + 0.93	5.04 + 0.63	5.51 + 0.49	<0.001
Day 20	3.09 + 0.18	6.09 + 0.49	6.24 + 0.36	4.0 + 0.35	4.5 + 0.66	<0.001

Immunohistochemical Results

For the control group, there is a highly significant decrement in DA receptors ($p < 0.001$) in comparison with the induced
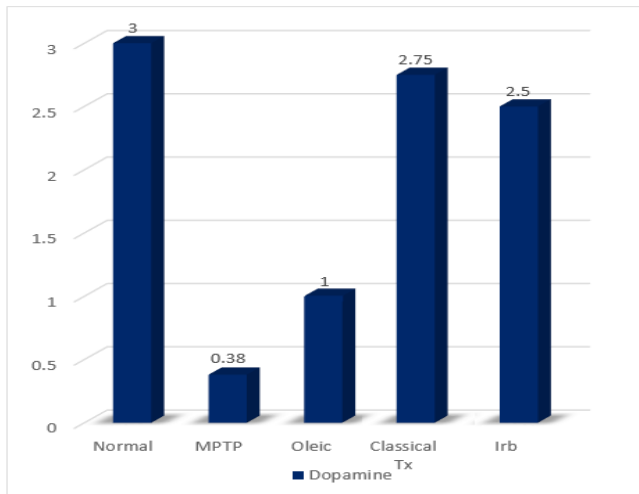
Figure 2: Dopamine receptor, caspase-3 & MDA in all study groups

Figure 3: Dopamine in all study groups

group (MPTP) group, besides no significant difference between MPTP group with an oleic acid group, a furthermore high significant decrease in DA receptor for Classical treatment (L-dopa/Carbidopa), and Irb in compare to MPTP group ($p < 0.001$) shown in Table 2 and Figure 2 and 4).

The DA significantly decreased in MPTP group in comparison to the control group ($p < 0.001$), while no significant difference in DA in oleic compared with MPTP, on the other hand, DA in L-dopa/Carbidopa and Irb significantly increased in comparison to the MPTP group ($p < 0.001$) viewed in Table 3 and Figures 3 and 5.

High significant increase in caspase-3 in MPTP group when compared with a control group ($P < 0.001$), no significant variance in caspase-3 in oleic and L-dopa/Carbidopa groups in comparison to MPTP group ($p > 0.05$), and for Irb group high significant decrement in caspase-3 in comparison to MPTP group ($p < 0.001$) reviewed in Table 4 and Figures 2 and 6.

In Table 5 and Figure 2 and 7 there is high significant elevation in MDA in MPTP group in comparison to the control group ($p < 0.001$), while no difference in MDA between MPTP and oleic ($p > 0.05$), meanwhile there is high significant decrement in MDA for L-dopa/Carbidopa and Irb in comparison with MPTP groups ($p < 0.001$)

DISCUSSION

PD is a common CNS degenerative disorder with tremor muscle rigidity. Motor instability, the pathology of PD are DA neurons collapse in the SNpc and leading to the loss of dopaminergic terminals in the striatum that cause a sharp reduction in the neurotransmitter DA levels. The formation of lewy bodies (LB) in cells, besides neuroinflammation, is a main pathological mechanism of PD. It is also a major target for PD treatment,[3,4] which pathway is through microglia-

Figure 4: Immunohistochemical expression of dopamine receptor in nigrostriata (A) control tissue shown low intensity of dopamine receptor (10X), (B) Induce tissue and (C) Oleic acid treated tissue shown high intensity of dopamine receptor (10X), (D) L-dopa/Carbidopa and (E) Irbesartan treated tissue shown low intensity of dopamine receptor (10X)

Table 2: Dopamine receptor level in all groups

Group	DA receptor	p-value
Control	0.0 ± 0.0^b	<0.001
MPTP	2.63 ± 0.52^a	<0.001
Oleic oil	2.75 ± 0.46^b	> 0.05
L-dopa/Carbidopa	2.75 ± 0.46^b	<0.001
Irb	1.25 ± 0.46^b	<0.001

[a]Comparison with control group; comparison with MPTP group. DA receptor, Dopamine receptor; MPTP, 1methyl4phenyl1,2,3,6tetrahydropyridine; Irb, Irbesartan. Values are presented as means ± standard deviations.

Table 3: Dopamine level in all groups

Group	DA	P value
Control	3.0 ± 0.0^b	<0.001
MPTP	0.38 ± 0.52^a	<0.001
Oleic oil	0.67 ± 0.52^b	>0.05
L-dopa/Carbidopa	2.75 ± 0.46^b	<0.001
Irb	2.5 ± 0.53^b	<0.001

[a]Comparison with control group; comparison with MPTP group. DA, dopamine; MPTP, 1methyl4phenyl1,2,3,6tetrahydropyridine; EO, essential oil; VPP, vitex's polyphenols; Irb, Irbesartan. Values are presented as means ± standard deviations.

Table 4: Caspase-3 level in all groups

Group	Casp-3	p-value
Control	3.0 ± 0.0^b	<0.001
MPTP	0.38 ± 0.52	<0.001
Oleic oil	1.13 ± 0.35^b	>0.05
L-dopa/Carbidopa	2.75 ± 0.46^b	>0.05
Irb	2.5 ± 0.53^b	<0.001

a Comparison with a control group; bcomparison with MPTP group. Casp-3, caspase-3; MPTP, 1methyl4phenyl1,2,3,6tetrahydropyridine; Irb, Irbesartan. Values are presented as means ± standard deviations.

Table 5: Malondialdehyde level in all groups

| Group	MDA	p-value
Control	0.0 ± 0.0^b	<0.001
MPTP	2.0 ± 0.0	<0.001
Oleic oil	1.75 ± 0.46^b	>0.05
L-dopa/Carbidopa	0.0 ± 0.0^b	<0.001
Irb	0.0 ± 0.0^b	<0.001

[a]Comparison with control group; [b]comparison with MPTP group. MDA, malondialdehyde; MPTP, 1methyl4phenyl1,2,3,6tetrahydropyridine; Irb, Irbesartan. Values are presented as means ± standard deviations.
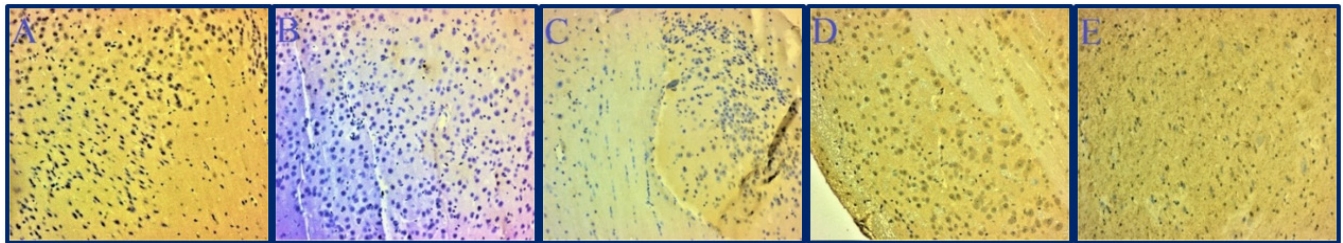
Figure 5: Immunohistochemical expression of dopamine in nigrostriata (A) control tissue shown high intensity of dopamine (10X), (B) Induce tissue and (C) Oleic acid treated tissue shown low intensity of dopamine (10X), (D) L-dopa/Carbidopa and (E) Irbesartan treated tissue shown high intensity of dopamine (10X)
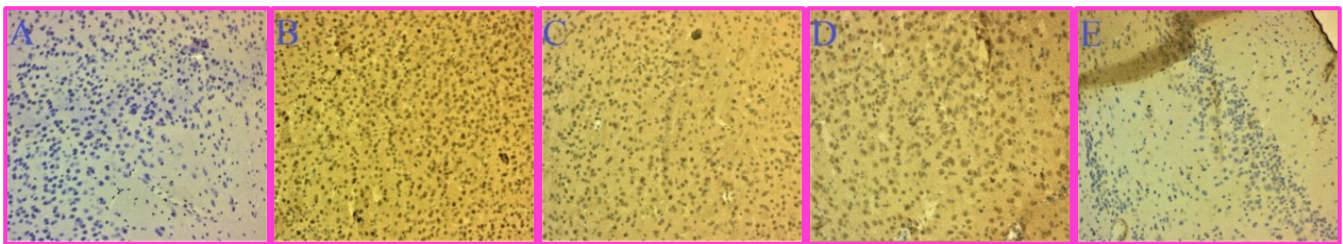
Figure 6: Immunohistochemical expression of Caspase-3 in nigrostriata (A) control tissue shown no intensity of caspase-3 (10X), (B) Induce tissue, (C) Oleic acid and (D) L-dopa/Carbidopa treated tissues shown high intensity of caspase-3 (10X) and (E) Irbesartan treated tissue shown low intensity of dopamine receptor (10X)
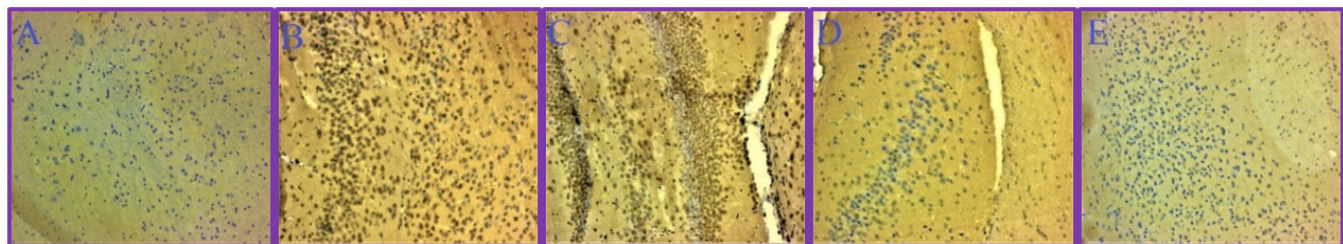
Figure 7: Immunohistochemical expression of malondialdehyde in nigrostriata (A) control tissue shown no intensity of malondialdehyde (10X), (B) Induce tissue and (C) Oleic acid-treated tissues shown high intensity of malondialdehyde (10X), (D) L-dopa/Carbidopa and (E) Irbesartan treated tissue shown low intensity of malondialdehyde (10X)

mediated neuroinflammation, oxidative stress-mediated mitochondrial dysfunction, and apoptosis also play a main etiologic part.[5,6] The present therapeutic strategy of PD relies mainly on Levodopa treatment, which can slow down disease progression to a limited degree with related side effects, yet no curative drug or agent able to hesitant PD progression.[37] The dietary herbal supplements have been efficaciously applied in the inhibition or protection of neuronal loss and are valuable agents for preventing tPD'sinflammatory process.[38,39]

The major findings of the study are followings: treatment with tested agents decreased the behavioral disorder and progression of Parkinson disease in mice induced by MPTP, DA level increased and DA receptor down-regulated by the tested agents and tested Agents decreased the level of caspase-3 and MDA, which may result in the reduction of cell death of neurons.

The disease shows some clinical characteristics as motor dysfunction[40] along with bradykinesia, resting tremor, rigidity, and postural instability.[41]

The prominent cause of motor-related symptoms is loss of dopaminergic neurons within the SNpc via several biochemical pathways having oxidative stress, mitochondrial inhibition, inflammation, accumulation of iron, and anti-oxidants reduction.[40,42]

The study viewed no significant variation in time to reach the base of the bar on day 5 for all tested groups (p > 0.05). There were huge significant variation in time for the days 10, 15, and 20 where (p < 0.001) shown in Table 1, since of the tested agents modulate the DA level and possess anti-oxidant and decrease the neuroinflammation, these finding agreed with the previous study for climbing pole test for assessing the behavioral changes.[30]

DA neurons are found in the SNpc; these neurons connect with other neurons by releasing the neurotransmitter DA,[43] DA plays an essential role in brain activation,[44] these neurons increasingly damaged, and their loss results in a reduced level of DA available for neurotransmission.[45] MPTP reduces brain activity by decreasing the dopaminergic neurons which results in a disturbance in locomotor activity.[43]

DA receptor type 2 (DA-D2 rec) is a major target for PD treatment and is important for DA signaling pathways.[46] The decrease in DA and its metabolites levels is accompanied by an increased number of dopaminergic D2 receptors in the striatum[47] since the main damage of striatal innervation and compensatory modifications occurs involving elevated DA synthesis release the remaining neurons and up-regulation of postsynaptic DA receptors.[48]

Caspase-3 is a main initiator and one of the hallmark characteristics of apoptosis in DA neurons.[19] DA cell loss is related with the triggering of caspase-3 in PD.Caspase-3 triggering is the principal effector of the mitochondrial-dependent apoptotic pathway.[49] Caspase-3 signaling cascade stimulate apoptotic cell loss in the neuronal cells[50] so many studies aim to deactivate caspase-3 activity to prevent apoptotic cell death.[51]

The free radicals generated during stress, target membrane lipids that is simply interact with an O2 molecule to give a peroxy radical that is liable for lipid peroxidation.[52] MDA (the biomarker for lipid peroxidation) acts as a reactive aldehyde and is a stable end product of lipid peroxidation,[53] resulting in the collapse of DA neurons.[54]

MDA, which is the main product of lipid peroxidation[55] its breakdown and assessment is reflected to be a dependable marker of oxidative loss and markers of the endogenous anti-oxidant system,[56] PD present with an elevated level of MDA; also MDA level elevated in mice treated with MPTP.[57]

This local RAS involved in the regulation of modulation of intraneuronal dopamine levels of oxidative stress and neuroinflammatory response, the brain carries an independent local renin-angiotensin system (RAS), and AT1 and AT2 receptors are expressed in dopaminergic neurons,[58] and striatum express AT1 receptors,[59] also microglial cell express both AT1 and 2 receptors which AngII binding to AT1 receptor cause activation of glial cell and progress of inflammatory response, so blocking AT1 receptors by ARBs lead to upregulation of AT2 receptors besides activation of PRAR-γ lead to decrease the oxidative stress and inflammatory process.[60] Ang II induces apoptosis depending on mitochondrial activity through the activation of AT1 receptors,[61] beside it activate autophagy and stimulate apoptosis.[62]

Irb has high affinity to AT1 receptor blocking with no affinity for AT2 receptor because AT1 receptor involved in oxidative damage, increase the dopamine level by the neuroprotective effect of Irb through decreasing the oxidative stress and inflammatory process, also increasing the dopamine level leads to down-regulation of D2 receptors (reverse the D2 receptor up-regulation).[63]

Blocking of AT1 also inhibits the caspase-3 activation and decreases its level by preventing mitochondrial-dependent apoptotic signaling by blocking Ang II-induced apoptosis in dopaminergic neurons.[19]

Also, Irb has an anti-oxidant effect by decreasing oxidative stress and reducing the production of ROS and lowering the MDA level.[64,65]

The results obtained in Table 2 revealed that oleic acid group has no effect on the progression of disease, furthermore high significant difference in DA receptor for classical treatment (L-dopa/Carbidopa) & Irb in compare to MPTP group which indicate that Irb show neuroprotective effect to dopaminergic neurons by causing downregulation of D2-receptor.

Irb effect on D2 receptor level in the study is similar to the previous study.[63,66,67]

Results got for the DA shown in Table 3 is significantly decreased in MPTP group in comparison to control group, while no significant difference in DA in oleic compared with MPTP, on the other hand DA in L-dopa/Carbidopa and Irb significantly increased in comparison to the MPTP group, revealed the neuroprotective effect of Irb to DA neurons that lead to increase DA level which cause motor stability.

The study finding show increase in DA level by Irb agreed with the previous study.[68-70]

Results appeared in Table 4 to be a highly significant increment in caspase-3 in MPTP in comparison with control groups, no significant variance in caspase-3 in oleic and L-dopa/Carbidopa groups in comparison to MPTP group, and for Irb group high significant decrement in caspase-3 in comparison to MPTP group, which reveal the anti-apoptotic effect of Irb.

The study of Irb that leads to decrease caspase-3 level is in agreement with the previous study.[69,71,72]

In Table 5, results from preview that there is a highly significant elevation in MDA in MPTP group in comparison to control group, while no difference in MDA between MPTP and oleic, meanwhile there is a high significant decrement in MDA for L-dopa/Carbidopa & Irb in comparison with MPTP groups, that view the anti-oxidant activity of L-dopa/Carbidopa, EO, VPP and Irb.

The study results that Irb causes a decrease in MDA level are similar to the previous study results.[73-75]

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