BENHA MEDICAL JOURNAL bmj

**Print ISSN** 1110-208X.

**Online ISSN** 2357-0016

# Impact of Semaglutide and Safinamide versus Bromocriptine on Parkinsonism Induced in Rats

Mohamed E. Mansour, Rezk A. Sanad, Mary E. Yacoub,

Sara A. Mosa, Salwa A. Elgendy

Clinical Pharmacology Department, Faculty of Medicine Benha University, Egypt.

Corresponding to:
Dr. Sara A. Mosa.
Clinical Pharmacology Department, Faculty of Medicine Benha University, Egypt.
Email: saraawad1292@gmail.com

Received:
Accepted:

## Abstract:

**Background:** Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by motor impairments such as bradykinesia, rigidity, tremors, and postural instability. Oxidative stress, neuroinflammation, and dopaminergic neuronal loss- are key pathological features. **Aim:** This study aimed to evaluate and compare the neuroprotective effects of semaglutide and safinamide against bromocriptine in a rat model of PD. **Materials and Methods:** Parkinsonism was induced in rats using rotenone. Animals were divided into eight groups: control, DMSO, bromocriptine (2.5 mg/kg), semaglutide (0.62 mg/kg), safinamide (10 mg/kg), and their respective combinations. Biochemical assays were performed to measure malondialdehyde (MDA), glutathione (GSH), and dopamine levels. Inflammatory cytokines (TNF-α, IL-6) were analyzed. Behavioral tests assessed motor function. Histopathological and immunohistochemical evaluations, including caspase-3 expression, were conducted. **Results:** All treated groups showed significant improvements in oxidative stress parameters, dopamine restoration, and inflammatory cytokine reduction. Behavioral performance improved across all drug-treated groups, with combined therapies yielding superior outcomes. The bromocriptine + safinamide group exhibited the greatest neuroprotective and functional recovery effects. **Conclusion:** Bromocriptine, semaglutide, and safinamide- either alone or in combination- demonstrated therapeutic efficacy in rotenone-induced Parkinsonism in rats. Combination therapies- particularly bromocriptine + safinamide- showed enhanced neuroprotective benefits, suggesting a promising strategy for PD management.

**Key words:** Parkinsonism; Bromocriptine; Semaglutide; Safinamide.

## Introduction

It is anticipated that Parkinson's disease will double in prevalence over the next three decades, making it the second most prevalent neurodegenerative disease after Alzheimer's [(1)].

Parkinson's disease can be diagnosed by utilizing both motor and non-motor symptoms. Motor symptoms that distinguish Parkinson's diseases include rigidity, postural instability, bradykinesia/akinesia, and rest tremor (pill-rolling) [(2)].

Currently, L-DOPA is the most effective anti-parkinsonian drug obtainable and is regarded as the "gold standard" in Parkinson's disease therapy [(3)]. Bromocriptine, cabergoline and pergolide are among the dopamine agonists that are frequently used as monotherapy during the initial stages of Parkinson's disease to postpone the motor complications that are caused by levodopa [(4)].

For over three decades, bromocriptine has been extensively employed in clinical settings to address parkinsonism, hyperprolactinemia, pituitary adenomas, and galactorrhea [(5)].

Semaglutide is the sole GLP-1RA that is presently available in both subcutaneous and oral formulations. This represents the most recent authorization of a glucagon-like Peptide-1 receptor agonist (GLP-1RA) [(6)]. Additionally, it has demonstrated neuroprotective properties in both animal and human studies [(7)].

In the human brain, safinamide is a highly selective, reversible MAO-B inhibitor that inhibits MAO-B one thousand times more potently than MAO-A [(8)]. In addition to its ability to obstruct voltage-dependent Na+ and Ca2+ channels and impede glutamate release, safinamide also impacts both dopaminergic and glutaminergic systems [(9)].

**Aim of work:**

The objective of the current investigation is to assess the influence of semaglutide and safinamide on experimentally induced parkinsonism in rats in comparison to bromocriptine

## Materials & methods:

**Approval code :** MD 4-1-2023

This study is a preclinical, experimental animal study conducted on adult male albino rats. The experiments were performed at the pharmacology department of the Benha Faculty of Medicine, Egypt, from July 2023 to July 2024. Ethical approval was obtained from the institutional animal care and use committee (IACUC), with approval code:MD 4-1-2023. All procedures were performed in accordance with the guidelines for the care and use of laboratory animals.

**A. Animals:**

At the commencement of the investigation, forty-eight adult male Sprague–Dawley rats, whose weight ranged from 150 to 200 g, were obtained from the Experimental Animal Breeding Farm, Helwan, Cairo, for use in the in vivo study. They were contained in a compartment that was kept at ambient temperature and was entirely ventilated in the pharmacology department of the Benha Faculty of Medicine. There, they were caged in groups of six for one week as part of the acclimatization process. Rats were supplied with water and a standard diet.

**B. Drugs and chemicals:**

Rotenone powder 1gram (Sigma-Aldrich Co-USA), saline (EL-Gomhouria Co., Egypt), semaglutide (powder) (Novo Nordisk., Denmark), safinamide (powder) (EVA pharmaceutical Co, Egypt), Bromocriptine (powder) (Amoun pharmaceutical Co, Egypt), interleukin 6 (IL-6) kits (MyBioSource, USA,Cat# MBS269892), tumor necrosis factor alpha (TNF-α) kits. (Cat# MBS2507393, MyBioSource, USA), malondialdehyde (MDA) and reduced glutathione assays are conducted at Bio-diagnostic in Egypt. (Bio-diagnostic, Egypt) Dopamine is detected in brain tissue preparations. Item number MBS779655 is included in the

category. The research utilized the following substances: anti-Caspase-3 (Dako, CA, USA), dimethyl sulfoxide (DMSO) (Sigma Chemical Co., USA), formalin, solution, neutral 10% formaline (El Gomhoria Pharmaceutical Chemical Co., ARE), urethane (Ethyl carbamat, white crystals) (Sigma Chemical Co., USA), and hematoxylin and eosin (E. Merk, Darmastadt, Germany).

**Induction of Parkinsonism:**

Rotenone (1.5 mg/kg/day; s.c.) is administered to rodents on a daily basis for a duration of 28 days to induce Parkinsonism [(10)].

The drug therapy started for four successive weeks after the end of induction of Parkinsonism [(11)].

**Drugs solvent:**

Bromocriptine [(12)], semaglutide [(13)] and safinamide [(14)] were dissolved in dimethyl sulfoxide (DMSO) and were given oral

**D. Experimental design:**

**Group I (control group):** This group received standard chow and tap water with no medication for 2 months.

**Group II (non-treated parkinsonism group):** Rotenone (1.5 mg/kg/day; s.c.) was administered once daily to rodents to induce Parkinsonism for twenty-eight consecutive days [(10)].

**Group III (DMSO Parkinsonism group):** This group given 0.5% DMSO containing water, oral for four successive weeks after induction of Parkinsonism [(12)].

**Group IV (Bromocriptine treated parkinsonism group):** This group given bromocriptine, oral in a dose 2.5 mg /kg /day [(15)].

**Group V (Semaglutide treated parkinsonism group):** This group given semaglutide, oral in a dose 0.62 mg/kg/day [(16)].

**Group VI (Safinamide treated parkinsonism group):** This group given safinamide, oral in a dose 10 mg /kg /day [(17)].

**Group VII (Bromocriptine + Semaglutide treated parkinsonism group):** This group given bromocriptine and semaglutide, oral in the same doses as above previous groups.

**Group VIII (Bromocriptine + Safinamide treated parkinsonism group):** This group given bromocriptine and safinamide, oral in same doses as above previous groups.

The selection of the appropriate dose was determined by pilot experiments and previously published studies.

**E. Body weight assessment:** Body weight assessed every week for all experimental groups by digital scale and all values recorded.

**F. Blood pressure assessment:** Measurement of blood pressure (BP) on the animal's tail [(18)].

**G. Behavioral tests:** Between 9:00 a.m. and 3:00 p.m., all behavioral procedures were administered in a silent room throughout the duration of the test.

**1. Open field test:** Assessment of locomotor behavior [(19)].

**2. Catalepsy test:** To assess muscular rigidity [(20)].

**3. Balance beam test:** Fine coordination and balance can be assessed [(21)].

Microcapillary tubes were employed to obtain blood samples from the retro-orbital venous plexus of rodents [(22)]. Samples of tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) were stored in opaque containers at -20 Ć for purposes of measurement.

After that, rodents were anesthetized with urethane (ethyl carbamate; 1.4 g/kg, i.p.) [(23)], and sacrificed for sample collection. The brains were immediately isolated and immersed in ice-cold saline. The midbrain homogenate was used to evaluate the biochemical parameters in nigral tissues in the midbrain, including malondialdehyde (MDA), glutathione (GSH), and dopamine. Brains from each cohort were collected and preserved in 10% formalin for histopathological and immunohistochemical evaluation of Caspase-3 [(24)].

**H. Biochemical essays:**

I. **Measurement of TNF- α and IL-6:** Use of a rodent enzyme-linked immunosorbent assay (ELISA) to detect TNF-α and interleukin 6 in rat serum [25,26].

II. **Measurement of Dopamine and oxidative stress markers (GSH and MDA) in mid brain homogenates:** Dopamine level measurement: ELISA kits [27]. To determine the concentrations of GSH and MDA in the brain, suitable assays were implemented through calorimetry [28].

**I. Histopathological analysis:** A histopathological assessment of specimens that have been stained with H&E [29].

**J. Immunohistochemistry:**

Immunostaining was conducted using the primary antibodies of rabbit anti-Caspase-3 and mouse anti-Fas, Immunohistochemistry was performed on paraffin sections using the avidin–biotin peroxidase method. Sections were incubated with rabbit anti-caspase-3 and mouse anti-Fas antibodies, then with the corresponding secondary antibodies, and the reaction was visualized with diaminobenzidine. Staining was evaluated by estimating the percentage of positive cells and their intensity [30].

**Statistical analysis:** The medical information included in a report. The statistical software for the social sciences (SPSS) version 26 was used for data analysis. The ANOVA test is implemented to compare the mean of quantitative data from more than two distinct groups in statistical analysis.

The P value is statistically significant if it is less than 0.05, while it is statistically insignificant if it is greater than 0.05. A P value of less than 0.01 is regarded as highly significant in all analyses.

## Results:

**Brm: Bromocriptine Sema: Semaglutide Safina: safinamide**

The significance conveyed by letters of different values [a (the highest value) and g (the lowest value)] .

**1. Assessment of the body weight:** Table (1)

**Table (1):** The effect of semaglutide and safinamide versus bromocriptine on body weight (gm) in different weeks:

| groups / weeks | G I (control) | G II (non-treated) | G III (DMSO) | G IV (Brm) | G V (Sema) | GVI (Safina) | G VII (Brm+ Sema) | GVIII (Brm+ Safina) |
|---|---|---|---|---|---|---|---|---|
| **1st week (start of the study)** | 195.00 [a] ± 7.72 | 199.33 [a] ± 8.59 | 198.50 [a] ± 5.39 | 196.83 [a] ± 4.49 | 191.83 [a] ± 7.14 | 193.33 [a] ± 9.71 | 193.83 [a] ± 5.49 | 191.83 [a] ± 5.56 |
| **4th week (end of induction)** | 258.67 [a] ± 6.86 | 209.17 [b] ± 8.01 | 209.33 [b] ± 7.53 | 210.67 [b] ± 10.03 | 208.33 [b] ± 7.71 | 209.00 [b] ± 10.12 | 207.33 [b] ± 8.98 | 207.67 [b] ± 9.37 |
| **8th week (end of the study)** | 281.00 [a] ± 2.83 | 220.67 [b] ± 5.75 | 218.50 [b] ± 6.75 | 185.50 [c] ± 4.37 | 158.67 [d] ± 1.97 | 219.67 [b] ± 6.02 | 131.83 [e] ± 2.14 | 185.50 [c] ± 4.37 |

Our study showed there were no significant difference in body weight between all groups at the first week of the study ,then there were significant decrease in body weight in all parkinsonism groups in comparison with control group p value< 0.01 , at the end of the study there were significant variations in body weight between groups as the following all groups showed significantly lower body weight than control group with the lowest result in bromocriptine +semaglutide treated group VII.

2. **Assessment of blood pressure:** Figure (1)

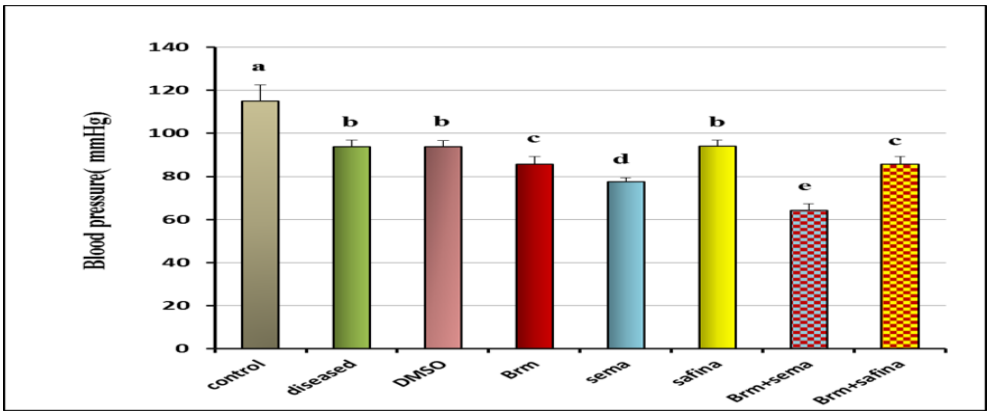



**Figure (1):** Histogram showing the effect of semaglutide and safinamide versus bromocriptine on blood pressure(mmHg).

According to blood pressure assessment, the results of the groups showed significant variations between groups. All groups showed significant lower blood pressure than control group with the lowest result in bromocriptine +semaglutide treated group VII.

3. **Assessment of behavioral tests:** Figures (2,3.4,5)

As regards behavioral tests, there were significant limitations of locomotor activity in all parkinsonism groups in comparison with control group also there were improvement in all treated groups in comparison with non-treated and DMSO groups with the best improvement in bromocriptine +safinamide treated group VIII.

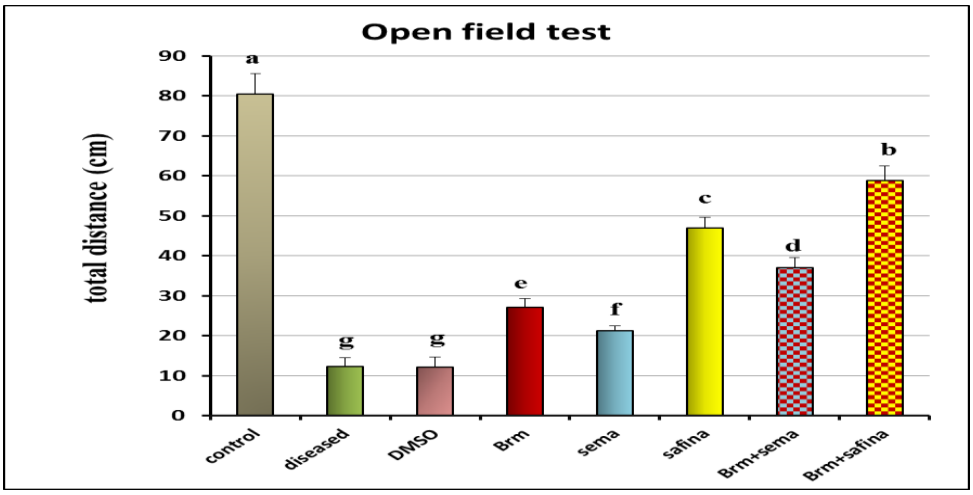



**Figure (2):** Histogram of assessing open field test.

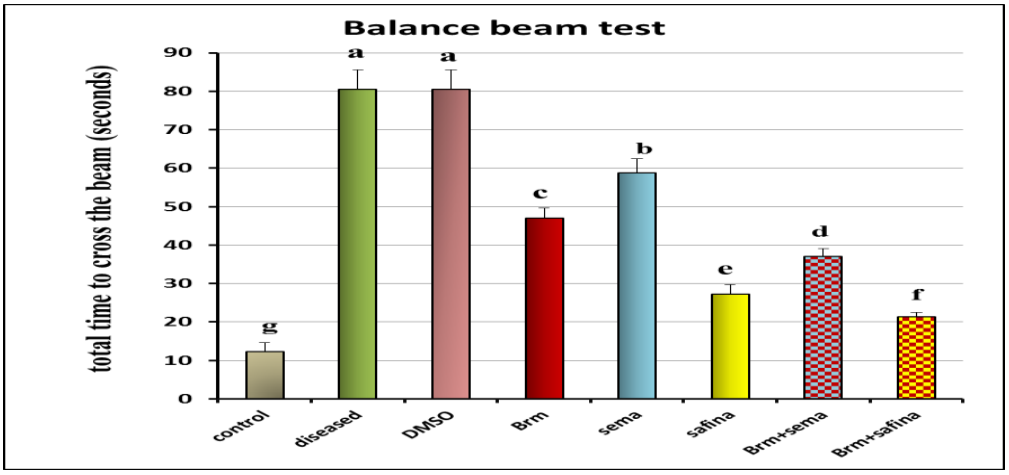



**Figure (3):** Histogram showing balance beam test assessing total time to cross the beam with seconds.

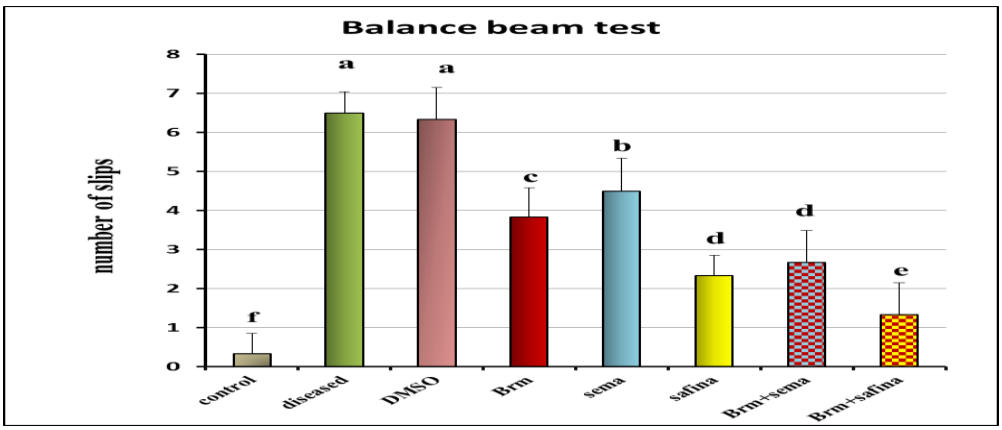



**Figure (4):** Histogram showing balance beam test assessing number of slips when crossing the beam.

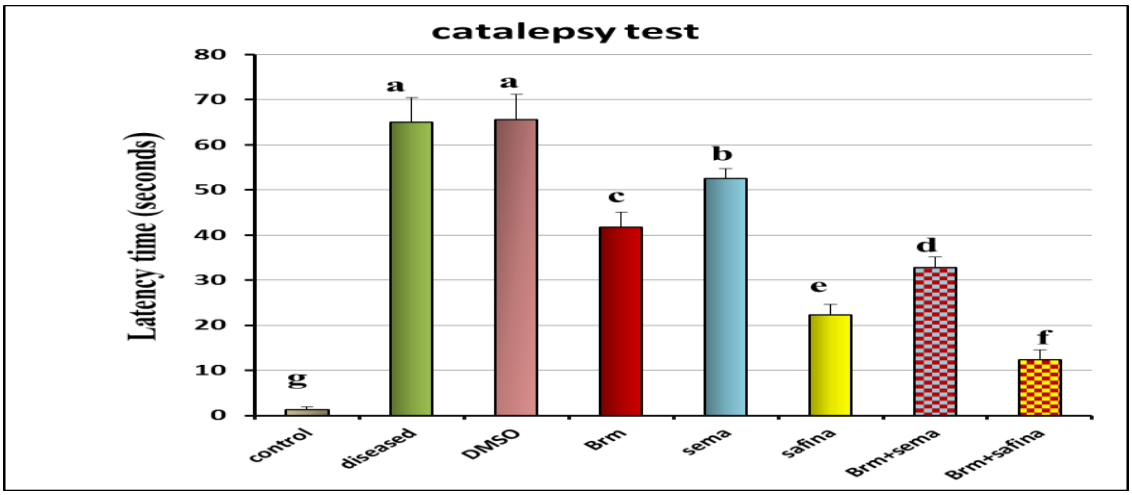



**Figure (5):** Histogram assessing catalepsy test.

**4. Measuerement of the serum inflammatory cytokines: Table (2)**

The inflammatory cytokines TNF-α and IL6 exhibited an increase in levels in the non-treated and DMSO groups, while their levels were significantly reduced in all treated groups p value< 0.01. The bromocriptine + safinamide treated group VIII exhibited the most favorable outcome.

**Table (2):** The effect of semaglutide and safinamide versus bromocriptine on serum inflammatory cytokines (serum TNF-α and IL-6):

| groups / weeks | G I (control) | G II (non-treated) | G III (DMSO) | G IV (Brm) | G V (Sema) | GVI (Safina) | G VII (Brm+ Sema) | GVIII (Brm+ Safina) |
|---|---|---|---|---|---|---|---|---|
| **TNF (pg/ml)** | 142.81 $^{f}$ ± 14.70 | 589.24 $^{a}$ ± 50.80 | 595.75 $^{a}$ ± 49.60 | 360.86 $^{c}$ ± 36.64 | 438.63 $^{b}$ ± 42.57 | 267.05 $^{d}$ ± 27.95 | 424.62 $^{d}$ ± 26.55 | 192.75 $^{e}$ ± 19.42 |
| **IL-6 (pg/ml)** | 40.55 $^{f}$ ± 3.97 | 179.07 $^{a}$ ± 14.21 | 185.16 $^{a}$ ± 13.40 | 133.42 $^{c}$ ± 10.73 | 152.08 $^{b}$ ± 12.10 | 97.18 $^{d}$ ± 7.45 | 85.57 $^{d}$ ± 7.23 | 58.35 $^{e}$ ± 6.05 |

**5. Measurement of Dopamine and oxidative stress markers in mid brain homogenate: Table (3)**

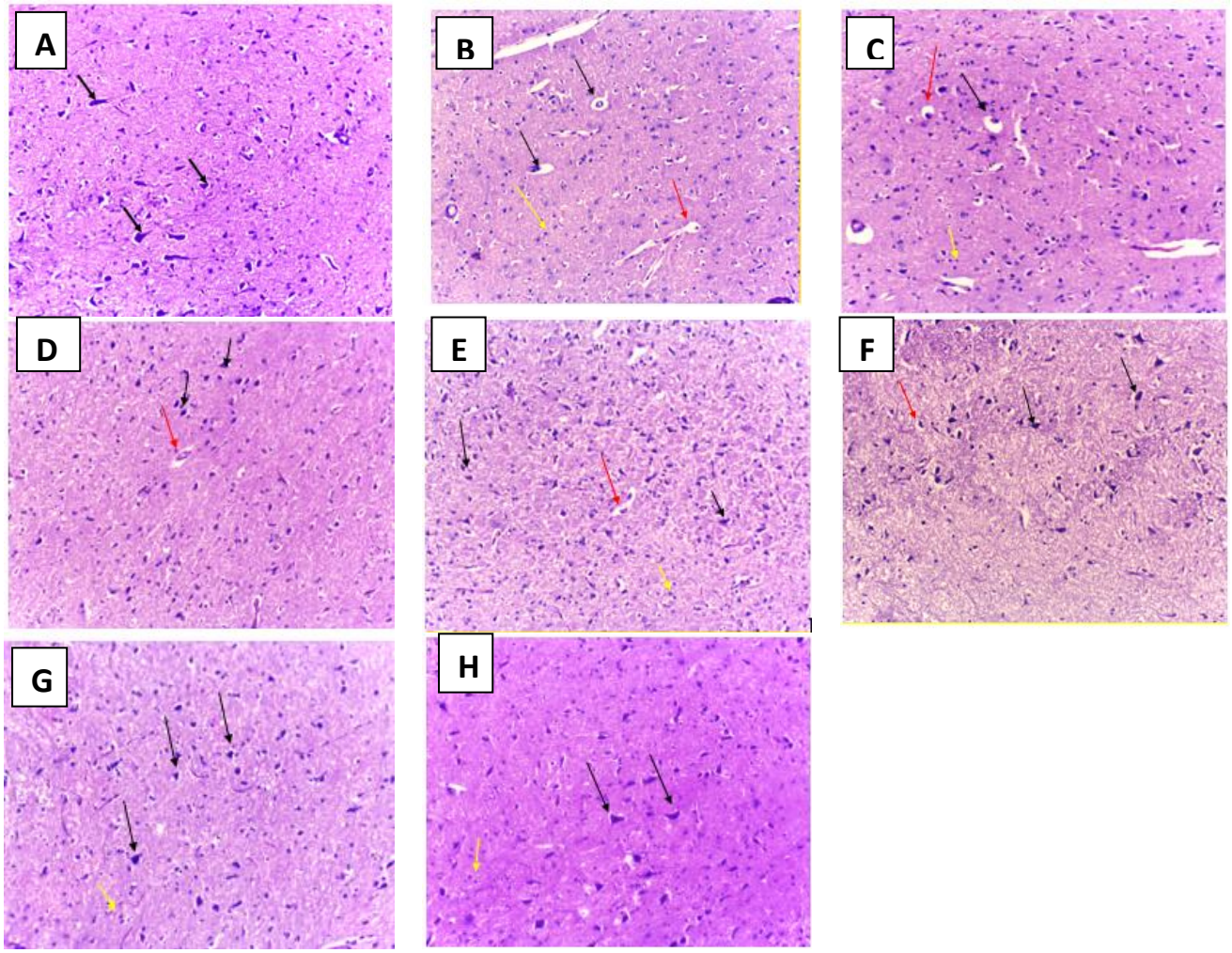
**Table (3):** The effect of semaglutide and safinamide versus bromocriptine on dopamine and oxidative stress markers (MDA) and GSH) in mid brain homogenate:

| groups / weeks | G I (control) | G II (non-treated) | G III (DMSO) | G IV (Brm) | G V (Sema) | GVI (Safina) | G VII (Brm+ Sema) | GVIII (Brm+ Safina) |
|---|---|---|---|---|---|---|---|---|
| **Dopamine (ng/l)** | 62.14 $^{a}$ ± 6.12 | 17.09 $^{f}$ ± 2.94 | 18.36 $^{f}$ ± 2.87 | 32.26 $^{d}$ ± 4.12 | 25.39 $^{e}$ ± 2.99 | 43.29 $^{c}$ ± 5.17 | 35.47 $^{d}$ ± 4.48 | 50.21 $^{b}$ ± 5.36 |
| **GSH (mg/g tissue)** | 6.79 $^{a}$ ± 0.86 | 2.63 $^{g}$ ± 0.30 | 2.56 $^{g}$ ± 0.32 | 3.45 $^{f}$ ± 0.39 | 3.90 $^{e}$ ± 0.42 | 4.28 $^{d}$ ± 0.51 | 5.00 $^{c}$ ± 0.59 | 5.84 $^{b}$ ± 0.71 |
| **MDA (nM/g tissue)** | 122.33 $^{f}$ ± 13.82 | 327.15 $^{a}$ ± 35.17 | 342.49 $^{a}$ ± 35.52 | 279.37 $^{b}$ ± 28.93 | 243.07 $^{c}$ ± 25.89 | 201.22 $^{d}$ ± 22.98 | 185.10 $^{d}$ ± 22.17 | 160.25 $^{e}$ ± 17.44 |

As regards dopamine level in midbrain homogenates there was significant reduction in dopamine level in non-treated and DMSO groups while its level was significantly increased in all treated groups with the best result in bromocriptine + safinamide treated group VIII p value< 0.01

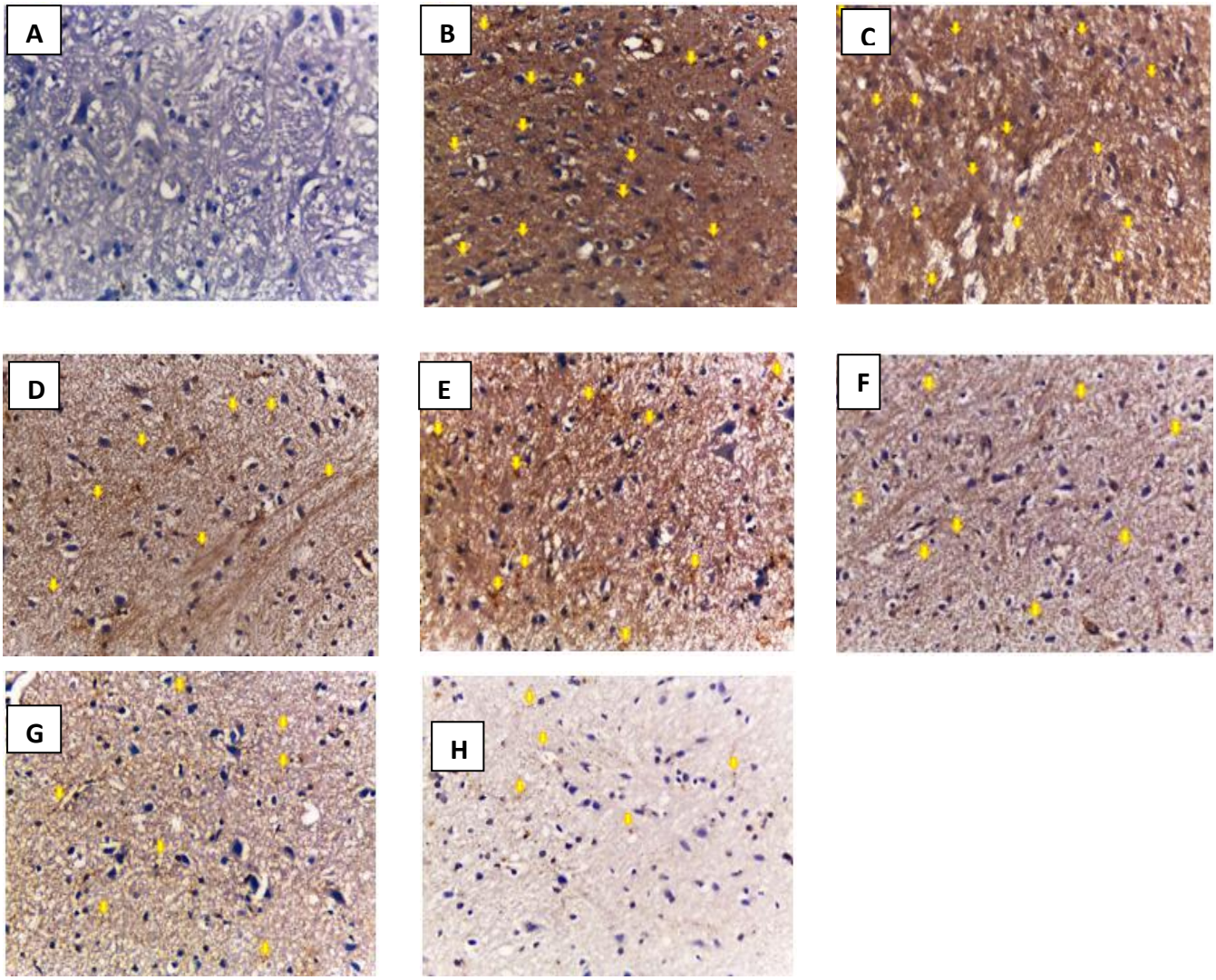
In the non-treated and DMSO groups, MDA levels increased significantly, whereas they decreased significantly in all threatened groups, particularly in the combination groups and other treated groups p value< 0.01. In addition, the GSH level was significantly decreased in the non-treated and DMSO groups, while it was significantly increased in all treated groups p value< 0.01. The bromocriptine + safinamide treated group VIII exhibited the most significant increase.

**Histopathological changes:**




Photomicrograph of midbrain sections (H&E, magnification ×200): **(A)** Group I (normal) shows preserved histoarchitecture with normal neurons (black arrow). **(B)** Group II (non-treated) reveals degenerated neurons (black arrow), pyknotic nuclei (red arrow), and inflammatory infiltrate (yellow arrow). **(C)** Group III (DMSO) shows similar degeneration and inflammation. **(D)** Group IV (Bromocriptine) exhibits partial neuronal recovery (black arrow) with some degeneration (red arrow). **(E)** Group V (Semaglutide) shows partial neuronal restoration (black arrow), some degeneration (red arrow), and inflammatory infiltrate (yellow arrow). **(F)** Group VI (Safinamide) reveals notable neuronal improvement (black arrow) with fewer degenerated cells (red arrow). **(G)** Group VII (Bromocriptine + Semaglutide) shows improved neurons (black arrow) and minimal inflammation (yellow arrow). **(H)** Group VIII (Bromocriptine + Safinamide) demonstrates neuronal restoration (black arrow) with mild inflammatory infiltrate (yellow arrow).

**Immunohistochemistry:**




**Immunohistochemical staining of midbrain for caspase-3 (magnification×400):**

**(A**) Group I (control): negative expression. **(B)** Group II (non-treated): strong positive expression. **(C)** Group III (DMSO): strong positive expression. **(D)** Group IV (Bromocriptine): mild positive expression. **(E)** Group V (Semaglutide): moderate positive expression. **(F)** Group VI (Safinamide): mild positive expression. **(G**) Group VII (Bromocriptine + Semaglutide): weak positive expression. **(H)** Group VIII (Bromocriptine + Safinamide): very weak positive expression (yellow arrow).

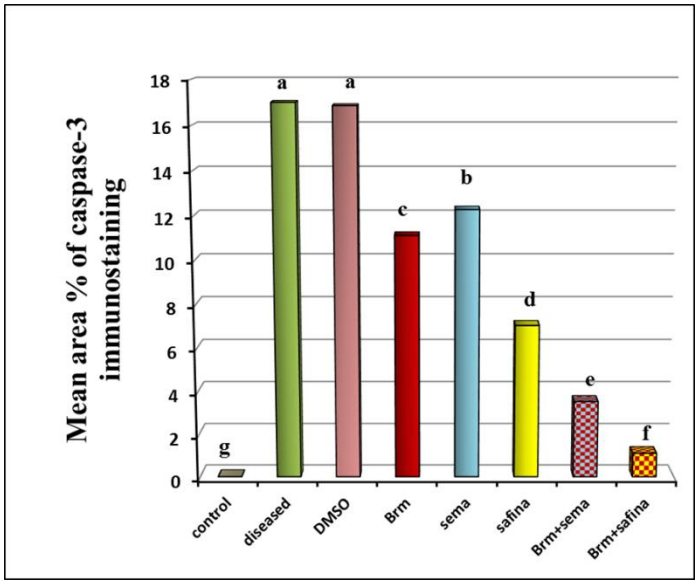



**Figure (٦):** Showing the mean area % of Caspase-3 immunostaining in all groups.

## Discussion:

The present results demonstrated a significant decrease in body weight in the non-treated, DMSO, and all treated groups when compared to the control group p value< 0.01. Among the treated groups, bromocriptine, semaglutide, and particularly their combination (bromocriptine + semaglutide) produced a pronounced reduction in body weight relative to the non-treated and DMSO groups, whereas safinamide alone did not exert a significant effect. The most marked decrease in body weight was observed in the bromocriptine + semaglutide group (Table 1). These findings confirm that safinamide does not influence body weight, which is consistent with earlier reports [31, 32] showing that bromocriptine and semaglutide reduce body weight, while another study [33] demonstrated that safinamide had no significant impact.

Weight loss observed in the parkinsonism groups can be attributed to disease-related factors. As reported by Kashihara [34], Parkinson's disease is frequently associated with appetite loss, which may arise from impaired olfaction and taste, gastrointestinal dysmotility, and depression. The anorectic effect of bromocriptine is linked to reduced hypothalamic neuropeptide Y (NPY) mRNA expression through D2 receptor activation, thereby counteracting the elevated NPY levels typically observed in obese models [35]. In addition, the weight-reducing mechanism of semaglutide has been attributed to its ability to suppress glucagon secretion, delay gastric emptying, reduce food intake, and decrease fat absorption, as documented by HamaSalih [36].

The present findings showed that blood pressure did not significantly decrease in the control group, whereas a significant reduction was observed in the non-treated, DMSO, and all treated groups. Safinamide (group VI) did not cause a significant decrease in blood pressure; however, bromocriptine, semaglutide, and particularly their combination induced a marked reduction compared with the non-treated and DMSO groups, with the greatest decrease observed in the bromocriptine + semaglutide group (Fig. 1).

These results suggest that safinamide has no hypotensive effect, which is consistent with previous reports indicating that hypotension is a common cardiovascular manifestation of PD [37]. The hypotension observed in the non-treated and DMSO

groups may be attributed to peripheral autonomic dysfunction, which develops early in the disease [38].

Consistent with our results, earlier studies demonstrated that bromocriptine and semaglutide reduced blood pressure [39, 40], whereas safinamide had no significant effect [41]. The hypotensive effect of bromocriptine has been attributed to inhibition of sympathetic nerve activity through D2 receptor activation, leading to reduced norepinephrine release [42]. Meanwhile, the blood pressure–lowering effect of semaglutide has been explained by its ability to promote natriuresis and diuresis in both experimental and clinical studies, an action associated with inhibition of the Na+/H+ exchanger 3 (NHE3) in the proximal tubules [43, 44].

Regarding the behavioral tests evaluated at the end of the four-week treatment period, all parkinsonian groups exhibited a significant reduction in locomotor activity compared with the control rats. Treatment with bromocriptine, semaglutide, or safinamide either as monotherapy or in combination resulted in significant improvement compared with the non-treated and DMSO groups p value< 0.01. with the greatest improvement observed in the bromocriptine + safinamide group (Group VIII).

Locomotor activity and mobility were lowest in the DMSO group (II) and the non-treated group (I), as previously reported [45].

Previous studies [46, 47] are consistent with our findings, showing that chronic administration of rotenone for three weeks markedly impairs cognitive and motor function. The present results also revealed a significant decrease in dopamine concentration p value< 0.01. which is consistent with the proposed mechanism underlying neurobehavioral impairment [48].

In agreement with earlier research [49, 50, 51], bromocriptine, semaglutide, and safinamide- respectively- were each able to improve motor behavior, supporting the current findings.

The mechanism underlying this improvement has been clarified by several studies. Bromocriptine alleviates bradykinesia associated with dopaminergic nigrostriatal degeneration by directly stimulating striatal dopamine D2 receptors, thereby enhancing locomotion [52]. Similarly, semaglutide was shown to improve motor behavior by attenuating dopamine depletion in nigrostriatal neurons [53]. Moreover, safinamide has been reported to exert beneficial effects on motor symptoms [54], which supports the observed improvements across all behavioral tests in the present model.

The present data demonstrated a significant increase in the levels of the inflammatory cytokines TNF-α and IL-6 in the non-treated and DMSO groups p value< 0.01. whereas their levels were markedly reduced in all treated groups (bromocriptine, semaglutide, and safinamide), either as monotherapy or in combination. The most pronounced reduction was observed in the bromocriptine + safinamide group (VIII). Both the non-treated and DMSO groups exhibited the highest levels of TNF-α and IL-6, consistent with previous findings [55] that identified rotenone as a key contributor to the pathogenesis of PD due to its selective toxicity toward nigrostriatal dopaminergic neurons. This aligns with evidence [56] that rotenone induces neuroinflammatory responses and microglial activation, leading to the release of cytotoxic cytokines, including IL-6 and TNF-α, which contribute to dopaminergic neuronal degeneration.

In agreement with our results, earlier studies [57, 58, 59] confirmed that bromocriptine, semaglutide, and safinamide respectively ameliorated serum IL-6 and TNF-α levels. The anti-inflammatory effect of bromocriptine has been attributed to its action on dopamine D2 receptors (Drd2), which suppress the transcription of pro-inflammatory cytokines, as demonstrated by Du et al., [60]. Similarly, GLP-1 receptor agonists, such as semaglutide, inhibit the production of pro-inflammatory cytokines,

adhesion molecules, and chemokines by attenuating NF-κB signaling, thereby reducing systemic inflammation [61]. Regarding safinamide, its ability to lower IL-6 and TNF-α is associated with its modulation of glutamate release, inhibition of $Ca^{2+}$ channel activity, and blockade of voltage-dependent $Na^{+}$ channels, ultimately reducing excitotoxicity and subsequent inflammatory mediator transcription, as confirmed by Blair & Dhillon [62].

Our investigation revealed that daily administration of rotenone for 28 days resulted in a significant elevation of MDA levels and a marked reduction in GSH levels in the midbrain homogenates of the non-treated and DMSO groups p value< 0.01. p value< 0.01. In contrast, treatment with bromocriptine, semaglutide, or safinamide either individually or in combination significantly decreased MDA levels and increased GSH levels p value< 0.01. p value< 0.01. with the most favorable outcomes observed in the bromocriptine + safinamide combination group (VIII). Consistent with our findings, previous studies [63] have confirmed that rotenone alters oxidative stress markers, including increased MDA and decreased GSH.

The underlying mechanism of rotenone toxicity is primarily related to the inhibition of mitochondrial complex I, leading to impaired ATP synthesis [64]. Additionally, rotenone suppresses the activity of antioxidant enzymes and promotes excessive production of reactive oxygen species (ROS), thereby exacerbating oxidative damage [65]. In agreement with the present results, earlier reports [66, 67, 68] demonstrated that bromocriptine, semaglutide, and safinamide, respectively, reduced MDA levels and restored GSH levels.

The antioxidant action of bromocriptine has been attributed to its ability to scavenge free radicals and inhibit their formation by enhancing the expression of nuclear factor erythroid 2–related factor 2 (Nrf2) [69]. Semaglutide has been shown to improve mitochondrial function and reduce oxidative stress [70], and its administration was further associated with upregulation of proteins involved in the Nrf2 signaling pathway [71, 72]. Similarly, safinamide has been reported to decrease microglial superoxide production and enhance glutathione synthesis, thereby exerting neuroprotective effects against axonal degeneration via modulation of ion channel activity [73].

In the present study, dopamine levels in the midbrain homogenates were markedly reduced in the non-treated and DMSO groups, whereas all treatment groups bromocriptine, semaglutide, and safinamide- whether administered individually or in combination- showed a significant restoration of dopamine levels. The highest dopamine concentration was detected in the bromocriptine + safinamide combination group (VIII). Rotenone is well known to selectively damage the nigrostriatal dopaminergic system when administered systemically, as it readily crosses cell membranes and inhibits mitochondrial complex I [74]. In line with our findings, previous studies [75,76,77] confirmed that bromocriptine, semaglutide, and safinamide each enhanced dopamine levels.

The effect of bromocriptine on dopamine preservation is attributed to stimulation of D2 receptors, which activates the MAPK and PI3K signaling cascades. This, in turn, upregulates the anti-apoptotic protein Bcl-2, thereby conferring protection to dopaminergic neurons [78]. Semaglutide has been reported to mitigate dopaminergic neuronal loss by reducing microgliosis and astrogliosis in experimental models, thus attenuating chronic inflammation [72], which is consistent with our findings. However, in contrast to our results, a previous report [53] observed that semaglutide produced only a non-significant increase in dopamine levels compared with diseased controls.

The neuroprotective effects of safinamide are largely related to its inhibition of

monoamine oxidase-B (MAO-B), thereby reducing dopamine catabolism. Elevated MAO-B activity is associated with increased production of hydrogen peroxide ($H_2O_2$), which contributes to neuronal degeneration and oxidative stress. Thus, MAO-B inhibition by safinamide represents a promising therapeutic strategy for maintaining dopamine levels, alleviating symptoms, and providing neuroprotection in Parkinson's disease [(79)].

## Conclusion:

Bromocriptine, semaglutide and safinamide improved parkinsonism symptoms. The combinations between drugs showed better result than single therapy thus could be used as new lines in treatment in cases of parkinsonism.

**Sources of funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

## References

(1) Tolosa, E., Garrido, A., Scholz, S. W., & Poewe, W. Challenges in the diagnosis of Parkinson's disease. *The Lancet Neurology*, (2021). *20*(5), 385-397.

(2) Balestrino, R., & Schapira, A. H. V. Parkinson disease. European journal of neurology, (2020). 27(1), 27-42.

(3) Bogetofte, H., Alamyar, A., Blaabjerg, M., & Meyer, M. Levodopa therapy for Parkinson's disease: history, current status and perspectives. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders), (2020). 19(8), 572-583.

(4) Luo, D., Reith, M., & Dutta, A. K. Dopamine agonists in treatment of Parkinson's disease: an overview. Diagnosis and Management in Parkinson's Disease, (2020).1, 445-460.

(5) Ashhar, M. U., Kumar, S., Ali, J., & Baboota, S. CCRD based development of bromocriptine and glutathione nanoemulsion tailored ultrasonically for the combined anti-parkinson effect. Chemistry and Physics of Lipids, (2021). 235, 105035.

(6) Smits, M. M., & Van Raalte, D. H. Safety of semaglutide. Frontiers in endocrinology, (2021). 12, 645563.

(7) Mahapatra, M. K., Karuppasamy, M., & Sahoo, B. M. Therapeutic potential of semaglutide, a newer GLP-1 receptor agonist, in abating obesity, non-alcoholic steatohepatitis and neurodegenerative diseases: A narrative review. Pharmaceutical Research, (2022). 39(6), 1233-1248.

(8) Jost, W. H. A critical appraisal of MAO-B inhibitors in the treatment of Parkinson's disease. Journal of Neural Transmission, (2022). 129(5), 723-736.

(9) Espinoza-Vinces, C., Villino-Rodríguez, R., Atorrasagasti-Villar, A., Martí-Andrés, G., & Luquin, M. R.Impact of Safinamide on Patient-Reported Outcomes in Parkinson's disease. Patient Related Outcome Measures, (2023).14, 285-295.

(10) Sharma, N., Jamwal, S., & Kumar, P. Beneficial effect of antidepressants against rotenone induced Parkinsonism like symptoms in rats. Pathophysiology, (2016). 23(2), 123-134

(11) Khadrawy, Y. A., Salem, A. M., El-Shamy, K. A., Ahmed, E. K.,Fadl, N. N., & Hosny, E. N. Neuroprotective and therapeutic effect of caffeine on the rat model of Parkinson's disease induced by rotenone. Journal of Dietary Supplements, (2017). 14(5), 553-572.

(12) Pathan, S. R., & Ansari, I. M. T. I. Y. A. Z. Studies on sensitivty of zebrafish as a model for parkinson's disease: comparison with mice model. Asian J Pharm Clin Res, (2018).11(11), 162-8.

(13) Xiao, X., Zhang, L., Ni, M., Liu, X., Xing, L., Wu, L., et al., Enhanced oral and pulmonary delivery of biomacromolecules via amplified transporter targeting. Journal of Controlled Release, (2024). (370), 152-167.

(14) Gu, X., Zhang, G., Qin, Z., Yin, M., Chen, W., Zhang, Y., et al., Safinamide protects against amyloid β (Aβ)-induced oxidative stress and cellular senescence in M17 neuronal cells. Bioengineered, (2022). 13(1), 1921-1930.

(15) Uhuo, E. N., Nwuke, C. P., Oriaku, C. E., & Chilaka, J. O. Biochemical and hormonal studies of metoclopramide-induced hyperprolactinemic female rats administered ethanol Leaf Extract of Kigelia africana. Comparative Clinical Pathology, (2021). 30(3), 371-377.

(16) Yamada, Y., Katagiri, H., Hamamoto, Y., Deenadayalan, S., Navarria, A., Nishijima, K., et al., Dose-response, efficacy, and safety of oral semaglutide monotherapy in Japanese patients with type 2 diabetes (PIONEER 9): a 52-week, phase 2/3a, randomised, controlled trial. The Lancet Diabetes & Endocrinology, (2020). 8(5), 377-391.

(17) Nam, M. H., Park, J. H., Song, H. J., Choi, J. W., Kim, S., Jang, B.K., et al., KDS2010, a newly developed reversible MAO-B inhibitor, as an effective therapeutic candidate for Parkinson's disease. Neurotherapeutics, (2021). 18(3), 1729-1747.

(18) Pauline, M., Avadhany, S. T., & Maruthy, K. N. Non invasive measurement of systolic blood pressure in rats: a simple technique. Al Ameen Journal of Medical Science, (2011).4(4), 365-369.

(19) Campos, F. L., Carvalho, M. M., Cristovão, A. C., Je, G., Baltazar, G., Salgado, A. J. & Sousa, N. Rodent models of Parkinson's disease: beyond the motor symptomatology. Frontiers in behavioral neuroscience, (2013). 7, 175.

(20) Luciani, K. R., Frie, J. A., & Khokhar, J. Y. An open source automated bar test for measuring catalepsy in rats. Eneuro, (2020). 7,3.

(21) Luong, T. N., Carlisle, H. J., Southwell, A., & Patterson, P. H. Assessment of motor balance and coordination in mice using the balance beam. Journal of visualized experiments: JoVE, (2011). (49), 2376.

(22) Schemer, S. The blood morphology of laboratory animals.3rd ed. Philadelphia,USA: Davis company; (1967):42.

(23) Field, K. J., White, W. J., & Lang, C. M. Anaesthetic effects of chloral hydrate, pentobarbitone and urethane in adult male rats. Laboratory Animals, (1993).27(3), 258–26.

(24) Badawi, G. A., Abd El Fattah, M. A., Zaki, H. F. Sitagliptin and liraglutide reversed nigrostriatal degeneration of rodent brain in rotenone-induced Parkinson's disease. Inflammopharmacology, (2017).25(3), 369-382.

(25) Kasumagic-Halilovic, E.; Prohic, A. and Cavaljuga, S. Tumor necrosis factor-alpha in patients with alopecia areata. Indian Journal of Dermatology, (2011): 56(5):494-6.

(26) Wojcik, B. M., Wrobleski, S. K., Hawley, A. E. Interleukin-6: a potential target for post-thrombotic syndrome. Annals of vascular surgery, (2011). 25(2), 229-239.

(27) El-Horany, H. E., El-latif, R. N. A., ElBatsh, M. M. Ameliorative effect of quercetin on neurochemical and behavioral deficits in rotenone rat model of Parkinson's disease: modulating autophagy (quercetin on experimental Parkinson's disease). Journal of biochemical and molecular toxicology, (2016). 30(7), 360-369.

(28) Ahmed, M. A., Ahmed, H. I., & El-Morsy, E. M. Melatonin protects against diazinon-induced neurobehavioral es in rats. Neurochemical research, (2013). 38(10), 2227-2236.

(29) Badawi, G. A., Abd El Fattah, M. A., & Zaki, H. F. Sitagliptin and liraglutide modulate L-dopa effect and attenuate dyskinetic movements in rotenone-lesioned rats. Neurotoxicity Research, (2019). 35(3), 635-653.

(30) Mandal, D., Mazumder, A., Das, P., Kundu, M., & Basu, J. Fas-, caspase 8-, and caspase 3-dependent signaling regulates the activity of the aminophospholipid translocase and phosphatidylserine externalization in human erythrocytes. Journal of Biological Chemistry, (2005).280(47), 39460-39467.

(31) Rizij, F. A., Al-lami, H. C. A., & Hussein, A. A. Effect of Bromocriptine on Anthropometric, Metabolic and Inflammatory Parameters in Obese Women. University of Thi-Qar Journal Of Medicine, (2018). 16(2), 1-11.

(32) Basha, S. M., Essawy, M. M., Ahmed, N. M., & Shawky, H. A. Assessment of Semaglutide Therapy on Inflammatory Mediators in Diabetic Rats with Experimental Periodontitis. Egyptian Dental Journal, (2024). 70(4), 3367-3377.

(33) Blair, H. A., & Dhillon, S. Safinamide: a review in Parkinson's disease. CNS drugs, (2017). 31, 169-176.

(34) Kashihara, K. Weight loss in Parkinson's disease. Journal of neurology, (2006). 253, vii38-vii41.

(35) Kuo, D. Y. Co-administration of dopamine D 1 and D 2 agonists additively decreases daily food intake, body weight and hypothalamic neuropeptide Y level in rats. Journal of Biomedical Science, (2002). 9, 126-132.

(36) HamaSalih, R. M. Effects of Semaglutide in Doxorubicin-Induced Cardiac Toxicity in Wistar Albino Rats. Cancer Management and Research, (2024).16, 731-740.

(37) Chen, Z., Li, G., & Liu, J. Autonomic dysfunction in Parkinson's disease: Implications for pathophysiology, diagnosis, and treatment. Neurobiology of disease, (2020). 134, 104700.

(38) Senard, J. M., Brefel-Courbon, C., Rascol, O., & Montastruc, J. L. Orthostatic hypotension in patients with Parkinson's disease: pathophysiology and management. Drugs & aging, (2001). 18, 495-505.

(39) Cincotta, A. H., Cersosimo, E., Alatrach, M., Ezrokhi, M., Agyin, C., Adams, J., et al., Bromocriptine-QR therapy reduces sympathetic tone and ameliorates a pro-oxidative/pro-inflammatory phenotype in peripheral blood mononuclear cells and

plasma of type 2 diabetes subjects. International journal of molecular sciences, (2022). 23(16), 8851.

(40) Wu, W., Tong, H. M., Li, Y. S., & Cui, J. The effect of semaglutide on blood pressure in patients with type-2 diabetes: a systematic review and meta-analysis. Endocrine, (2024). 83(3), 571-584.

(41) Stocchi, F., Vacca, L., Grassini, P., Tomino, C., Caminiti, G., Casali, M., et al., Overnight switch from rasagiline to safinamide in Parkinson's disease patients with motor fluctuations: a tolerability and safety study. European Journal of Neurology, (2021). 28(1), 349-354.

(42) Mannelli, M., Lazzeri, C., Ianni, L., La Villa, G., Pupilli, C., Bellini, F., et al., Dopamine and sympathoadrenal activity in man. Clinical and experimental hypertension (New York, N.Y. : 1993), (1997). 19(1-2), 163–179.

(43) Jensen, E. P., Poulsen, S. S., Kissow, H., Holstein-Rathlou, N. H., Deacon, C. F., Jensen, B. L. et al., Activation of GLP-1 receptors on vascular smooth muscle cells reduces the autoregulatory response in afferent arterioles and increases renal blood flow. American Journal of Physiology-Renal Physiology, (2015). 308(8), F867-F877.

(44) Kawanami, D., & Takashi, Y. GLP-1 receptor agonists in diabetic kidney disease: from clinical outcomes to mechanisms. Frontiers in Pharmacology, (2020). 11, 967.

(45) Madiha, S., Tabassum, S., Batool, Z., Liaquat, L., Sadir, S., Shahzad, S,et al., Assessment of gait dynamics in rotenone-induced rat model of Parkinson's disease by footprint method. Pakistan journal of pharmaceutical sciences, (2017). 30, 943-948.

(46) Sharma, N., & Nehru, B. Beneficial effect of vitamin E in rotenone induced model of PD: behavioural, neurochemical and biochemical study. Experimental neurobiology, (2013). 22(3), 214.

(47) Ramirez, A. D., Wong, S. K. F., & Menniti, F. S. Pramipexole inhibits MPTP toxicity in mice by dopamine D3 receptor dependent and independent mechanisms. European journal of pharmacology, (2003). 475(1-3), 29-35.

(48) MEKKEY, S. M., ABU RAGHIF, A. R., RIDHA ALKAFAJI, H. A., & HADI, N. R.The anti-Parkinson effects of Liraglutide in rat model of Rotenone induced Parkinsonism. International Journal of Pharmaceutical Research , (2020).12,449.

(49) Onaolapo, O. J., & Onaolapo, A. Y. Subchronic Oral Bromocriptine Methanesulfonate Enhances Open Field Novelty-Induced Behavior and Spatial Memory in Male Swiss Albino Mice. Neuroscience Journal, (2013). 2013(1), 948241.

(50) Meca, A. D., Boboc, I. K. S., Mititelu-Tartau, L., & Bogdan, M. Unlocking the Potential: Semaglutide's Impact on Alzheimer's and Parkinson's Disease in Animal Models. Current issues in molecular biology, (2024). 46(6), 5929–5949.

(51) Sciaccaluga, M., Mazzocchetti, P., Bastioli, G., Ghiglieri, V., Cardinale, A., Mosci, P., et al., Effects of safinamide on the glutamatergic striatal network in experimental Parkinson’s disease. Neuropharmacology, (2020).170, 108024.

(52) Hisahara, S., & Shimohama, S. Dopamine receptors and Parkinson's disease. International journal of medicinal chemistry, (2011). 2011, 403039.

(53) Zhang, L., Li, C., Zhang, Z., Zhang, Z., Jin, Q. Q., Li, L.,et al., DA5-CH and Semaglutide Protect against Neurodegeneration and Reduce α-Synuclein Levels in the 6-OHDA Parkinson’s Disease Rat Model. Parkinson’s Disease, (2022). 2022(1), 1428817.

(54) Müller, T. Safinamide for Treating Parkinson’s disease. NeuroPsychopharmacotherapy, (2020). 1,1-8.

(55) Yuan, Y. H., Sun, J. D., Wu, M. M., Hu, J. F., Peng, S. Y., & Chen, N. H.Rotenone could activate microglia through NFκB associated pathway. Neurochemical Research, (2013). 38, 1553-1560.

(56) Alabi, A. O., Ajayi, A. M., Ben-Azu, B., Bakre, A. G., & Umukoro, S. Methyl jasmonate abrogates rotenone-induced parkinsonian-like symptoms through inhibition of oxidative stress, release of pro-inflammatory cytokines, and down-regulation of immnopositive cells of NF-κB and α-synuclein expressions in mice. Neurotoxicology, (2019). 74, 172-183.

(57) Kutikuppala, L. V. S., Sharma, S., Chavan, M., Rangari, G., Misra, A. K., Innamuri, S. R., et al., Bromocriptine: does this drug of Parkinson’s disease have a role in managing cardiovascular diseases?. Annals of Medicine and Surgery, (2024). 86(2), 926-929.

(58) Shnaien, A. A., Mohammad, A. R., & Hassan, E. S. Neuroprotective effect of semaglutide in endotoxemia mouse model. Iranian Journal of War and Public Health, (2023).15(2), 1001-1007.

(59) Qian, L., Li, J. Z., Sun, X., Chen, J. B., Dai, Y., Huang, Q. X., et al., Safinamide prevents lipopolysaccharide (LPS)-induced

inflammation in macrophages by suppressing TLR4/NF-κB signaling. International Immunopharmacology, (2021). 96, 107712.

(60) Du, R. H., Zhou, Y., Xia, M. L., Lu, M., Ding, J. H., & Hu, G. α-Synuclein disrupts the anti-inflammatory role of Drd2 via interfering β-arrestin2-TAB1 interaction in astrocytes. Journal of neuroinflammation, (2018). 15, 1-13.

(61) Alharbi, S. H. Anti-inflammatory role of glucagon-like peptide 1 receptor agonists and its clinical implications. Therapeutic Advances in Endocrinology and Metabolism, (2024). 15, 20420188231222367.

(62) Blair, H. A., & Dhillon, S. Safinamide: a review in Parkinson's disease. CNS drugs, (2017). 31, 169-176.

(63) Jiang, X. W., Qiao, L., Feng, X. X., Liu, L., Wei, Q. W., Wang, X. W., et al., Rotenone induces nephrotoxicity in rats: oxidative damage and apoptosis. Toxicology mechanisms and methods, (2017). 27(7), 528-536.

(64) Heinz, S., Freyberger, A., Lawrenz, B., Schladt, L., Schmuck, G., & Ellinger-Ziegelbauer, H. Mechanistic investigations of the mitochondrial complex I inhibitor rotenone in the context of pharmacological and safety evaluation. Scientific reports, (2017). 7(1), 1-13.

(65) Radad, K., Al-Shraim, M., Al-Emam, A., Wang, F., Kranner, B., Rausch, W. D.,et al., Rotenone: From modelling to implication in Parkinson's disease. Folia neuropathologica, (2019) 57(4), 317-326.

(66) Kline, A. E., Massucci, J. L., Ma, X., Zafonte, R. D., & Edward Dixon, C. Bromocriptine reduces lipid peroxidation and enhances spatial learning and hippocampal neuron survival in a rodent model of focal brain trauma. Journal of neurotrauma, (2004). 21(12), 1712-1722.

(67) Botros, S. R., Matouk, A. I., Amin, A., & Heeba, G. H. Comparative effects of incretin-based therapy on doxorubicin-induced nephrotoxicity in rats: the role of SIRT1/Nrf2/NF-κB/TNF-α signaling pathways. Frontiers in Pharmacology, (2024). 15, 1353029.

(68) Wasan, H., Singh, D., Joshi, B., Sharma, U., Dinda, A. K., & Reeta, K. H. Post stroke safinamide treatment attenuates neurological damage by modulating autophagy and apoptosis in experimental model of stroke in rats. Molecular Neurobiology, (2021). 58(12), 6121-6135.

(69) Popovic, M., Janicijevic-Hudomal, S., Kaurinovic, B., Rasic, J., Trivic, S., & Vojnović, M. Antioxidant effects of some drugs on immobilization stress combined with cold restraint stress. Molecules, (2009). 14(11), 4505-4516.

(70) Alzubaidy, D. H. M., & Al-Isawi, Z. J. K. Investigating the Impact of Semaglutide on Hepatic Oxidative Stress in Obese Male Rats Induced by High-Fat Diet. Maaen Journal for Medical Sciences, (2024). 3(3), 3.

(71) Zhu, J., Wang, H., Yan, C., Li, B., & Chen, B. Ultrasound-targeted semaglutide-loaded PEG-nanoliposomes microbubble destruction protects diabetic cardiomyopathy by activating PI3K/Akt/Nrf2 signaling pathway. Heliyon, (2023). 9,9.

(72) Liu, D. X., Zhao, C. S., Wei, X. N., Ma, Y. P., & Wu, J. K. Protects against 6-OHDA Toxicity by Enhancing Autophagy and Inhibiting Oxidative Stress. Parkinson's Disease, (2022). 2022(1), 6813017.

(73) Morsali, D., Bechtold, D., Lee, W., Chauhdry, S., Palchaudhuri, U., Hassoon, P.,et al., Safinamide and flecainide protect axons and reduce microglial activation in models of multiple sclerosis. Brain : a journal of neurology, (2013) 136(Pt 4), 1067–1082.

(74) Xiong, N., Long, X., Xiong, J., Jia, M., Chen, C., Huang, J., et al., Mitochondrial complex I inhibitor rotenone-induced toxicity and its potential mechanisms in Parkinson's disease models. Critical reviews in toxicology, (2012). 42(7), 613-632.

(75) Ogawa, N., Tanaka, K. I., & Asanuma, M. Bromocriptine markedly suppresses levodopa-induced abnormal increase of dopamine turnover in the parkinsonian striatum. Neurochemical research, (2000). 25, 755-758.

(76) Zhang, L. Y., Jin, Q. Q., Hölscher, C. & Li, L. Glucagon-like peptide-1/glucose-dependent insulinotropic polypeptide dual receptor agonist DA-CH5 is superior to exendin-4 in protecting neurons in the 6-hydroxydopamine rat Parkinson model. Neural Regeneration Research, (2021). 16(8), 1660-1670.

(77) Chung, J. Y., Lee, J. W., Ryu, C. H., Min, H. K., Yoon, Y. J., Lim, M. J., et al., 1-[2-(4-Benzyloxyphenoxy) Ethyl] Imidazole inhibits monoamine oxidase B and protects against neuronal loss and behavioral impairment in rodent models of Parkinson's disease. Journal of Neuroscience Research, (2015). 93(8), 1267-1278.

(78) Mehta, A., Prabhakar, M., Kumar, P., Deshmukh, R., & Sharma, P. L. Excitotoxicity: bridge to various triggers in neurodegenerative disorders. European

journal of pharmacology, (2013). 698(1-3), 6-18.

(79) Mandel, S. A., Avramovich-Tirosh, Y., Reznichenko, L., Zheng, H., Weinreb, O.,

(80) Amit, T., et al., Multifunctional activities of green tea catechins in neuroprotection. Neurosignals, (2005). 14(1-2), 46-60.

**To cite this article:** Mohamed E. Mansour, Rezk A. Sanad, Mary E. Yacoub, Sara A. Mosa, Salwa A. Elgendy. Impact of Semaglutide and Safinamide versus Bromocriptine on Parkinsonism Induced in Rats. BMFJ XXX, DOI: 10.21608/bmfj.2025.388219.2438.