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Research article

The anti -Parkinson effects of Liraglutide in rat model of Rotenone induced Parkinsonism

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ABSTRACT

Using the classical dopamine-based anti-Parkinson drugs is associated with a broad spectrum of dilemmas that created the urgent need to include drugs that targets the other pathological events that accompanies the neuro degeneration process such as: oxidative stress, inflammation apoptosis, and formation of α-syncline aggregates, the various advantageous effects of Liraglutide lead to expect further neuroprotective effects. This study aims to investigate the anti-inflammatory, anti-oxidant, and anti-apoptotic effects of Liraglutide by measurement of dopamine (DA), tyrosine hydroxylase (TH), IL-1β, IL-6, cytochrome-c (Cyt-c), caspase-3 (Cas-3), myeloperoxidase (MPO), and α-syncline(SNCA) expression in midbrain samples of animal model of rotenone induced Parkinson disease (PD), and to perform neurobehavioral analysis and to compare it to that of pramipexole. 40 male albino rats were equally divided into: healthy control (no treatment), induction group (rotenone induced PD, 2.5 mg/kg IP every 48 hr for 20 days), pramipexole group (as in induction + pramipexole 1mg/kg orally after 30 min), Liraglutide group (as in induction+liraglutide50 µcg/kg Scafter 30 min).Compared to the induction group Liraglutide could significantly increase locomotion duration, reduce the duration to cross the balance beam, reduce the cataleptic state ($p<0.01$), it could also significantly increase DA content of midbrain tissue samples, and significantly reduceIL-1β, cas-3, Cyt–c, SNCA ($p<0.01$). Compared to pramipexole, Liraglutide could significantly increase open field locomotion duration, the duration to cross the balance beam and number of slips, and significantly reduce the duration to slip down the vertical pole ($p<0.01$), it revealed a significantly higher IL-1β tissue content, and SNCA expression ($p<0.01$). It was concluded that Liraglutide has potential anti-Parkinson effects that is worthy of further research.

Keywords: Liraglutide, Parkinson disease (PD).

INTRODUCTION:

Parkinson's disease (PD) is an intractable disease resulting in localized neuro degeneration of dopaminergic neurons of the substantianigra pars compact (SNc) [1]. PD is the second most common neurodegenerative disease, afflicting 1% of the population above the age of 65 [2]. A wide range of both environmental and genetic risk factors have been implicated in the pathogenesis of PD [3]. The multifactorial etiopathogensis of PD includes among others: mitochondrial dysfunction, oxidative/ nitrosamine stress, and inflammation. These events lead to the accumulation of abnormal or misfolded SNCA protein [4], the target of many therapeutic approaches [5]. Apoptosis as a main mechanism of neuronal death in Parkinson's disease is mediated by a number of initiator and executioner caspase [6]. Postmortem and in vitro studies illustrated elevated activity of cas-3 and increased expression of active cas-3 insubstantianigra pars compact [7]. Caspase inhibitors have also been shown to rescue neurons from death in cell models of Parkinson's disease [8]. SNCA has been suggested to lead to oxidative stress (OS) and the release of Cyt-c into the cytosol, in in vitro systems. Subsequent to its release into the

cytoplasm, Cyt-c interacts with pro-survival, ant apoptotic proteins, triggering mitochondria mediated apoptosis [9]. Neuroinflammation plays an important role in the pathogenesis of PD; its resolution is a process that allows for inflamed tissues to return to homeostasis [10]. Cytokines, Chemokines, and other inflammatory mediators are known to trigger microglial activation, potentially contributing to nigrostriatal pathway injury. As dopaminergic neurons express a wide range of cytokine and chemokine receptors, it has been suggested that they are responsive to these inflammatory mediators which are derived from or which activate microglia [11]. Elevated levels of the proinflammatory interleukins IL-1β, and IL-6 have also been detected in the striatum [12]. Some brain enzymes such as monoamine oxidase (MAO), and (TH), produce H2O2 as a normal byproduct of their activity, these enzymes are involved in DA metabolism and it seems that DA and its metabolites are involved in the production of reactive oxygen species (ROS) [13]. Some other exclusive ways, in which ROS is specifically produced in nigrostriatal DA ergic neurons, including reactive iron stored at neuromelanin (NM) and inflammation [14]. Liraglutide is soluble fatty acid acylated GLP-1 analog. It is approved in patients with type 2 DM who achieve inadequate control with diet and exercise and are receiving concurrent treatment with metformin, sulfonylurea's, or Thiazolidinediones [15]. Glucagon like peptide -1 (GLP-1) prove to pass the BBB through simple diffusion, so GLP-1 could induce neuritis outgrowth, and promotes proliferation and neuronal growth [16]. Type 2 diabetes mellitus shares pathophysiological mechanisms with PD and neurodegenerative dementias, such as central and peripheral insulin resistance that in turn results in altered autophagy, cell proliferation and increased inflammation [17]. This study aims to investigate the possible anti-Parkinson effects of Liraglutide.

MATERIALS AND METHODS

Preparation of animals

This is a case-control study that had recruited 40 healthy adult male Albino rats (Rattus Albinus), aged between 3-7 months, weighing 200-300 gm, purchased from the animal house of Iraqi Center of Cancer Research, they were housed 7 per cage for 1 month prior to the experiment and had been fed ad libitum and were allowed to drink tap water, in alternating 12 hr light/ 12 hr dark.

Ethical statement

All ethical themes of the studies on animals were considered carefully and the experimental protocol was approved by The International Review Board in The College of Medicine, of Al-Nahrain University.

Study design

The 40 healthy middle aged male rats were divided into 4 groups (10 animals / group)

Gr 1: The healthy control group, Gr 11: The induction group which was injected 2.5 mg/kg of rotenone solution IP (18), in a dark room, every 48 hr for 20 days, Gr III: The pramipexole group which was given oral pramipexole solution 1 mg/kg [19]. Gr VI: Liraglutide 50 μg/kg Sc [20], both of pramipexole and Liraglutide were given every 48 hr, 30 minutes after rotenone injection for 20 days.

Preparation of drugs

Pramipexole commercially available as oral tablets (Sifrol®), Liraglutide is commercially available in prefilled pen (Vectoza®) given by Sc. route.

Induction method

The pesticide rotenone has been shown to cause systemic inhibition of mitochondrial complex I activity, with consequent degeneration of dopamine neurons along the nigrostriatal pathway, as observed in PD [21] which can cause OS and lead to selective degeneration of striatal-nigral dopamine neurons. Besides, aggregation of SNCA and polyubiquitin, activation of astrocytic and microglial cells, inflammatory reaction, and neuronal apoptosis are all involved in the mechanisms of rotenone-evoked Parkinsonism [22]. Rotenone powder was dissolved in dimethyl sulfoxide (DMSO) to get a (0.00761 Mm solution) [18], then diluted in olive oil solution, fresh solution was prepared twice a week, before administration to rats the solution was vortexed to obtain a uniform mixture [23], the solution was kept refrigerated in dark glass container and wrapped with Aluminum foil, since rotenone is susceptible to rapid photodegredation [24].

Collection and preparation of samples

At the end of the experiment, or on day 21st the behavioral tests were performed the animals were anesthetized by a high dose of diethyl ether , to get midbrain samples which were subdivided into 2 groups, in the first the samples were weighed and kept in Eppendorf tube in dry ice before treatment with a phosphate buffer solution then

homogenized, centrifuged, allowed to settle and freeze overnight then, thawed, centrifuged again to get the supernatant on which the tests were performed to measure the biomarkers.The second group of the midbrain samples were preserved in 10% formalin solution for 48 hr then paraffin sections were prepared according to Bancroft and Gamble [25] as follows: fixation, dehydration, clearing, impregnation, embedding, sectioning, dew axing, hydration, and immunohistochemical staining then mounting.

Behavioral tests

All the behavioral tests were performed at the same room where the animals are housed, at a time between 10 A.M and 2 P.M, and the animals were trained on each test separately several times before being recorded. All the behavioral tests were performed at the same room where the animals are housed, at a time between 10 A.M and 2 P.M, and the animals were trained on each test separately several times before being recorded. These were: Open field locomotion test [26], Balance beam test [27], catalepsy test [28], and vertical pole test [29].

Biomarkers' measurement

All samples and reagents were brought to room temperature (25 ℃) before use. The samples were centrifuged again after thawing before the assay. The principle and procedure for each parameter measurement was done according to the manufacturer's instructions, based on ELISA principle to measure tissue concentration of DA, TH, IL-1β, IL-6, caspase-3, cytochrome-c. MPO was measured based on a photochemical reaction using a spectrophotometer.

Immunohistochemical study (IHC)

IHC was performed on formalin-fixed, paraffin-embedded tissue sections using a SNCA poly clonal antibody and a 2-step plus poly-HRP anti-rabbit/ mouse IgG detection system (with DAB solution). Histo Score is based on four immunohistochemistry categories reported in percent cells: negative (0), weak (1+), moderate (2+), and strongly (3+) stained membranes. In each case, a histo Score with a potential range of 0–300 was calculated as follows: Histo Score (H-score) = ((1×% weakly stained cells) + (2×% moderately stained cells) + (3×% strongly stained cells)) [30].

Statistical analysis

Data were collected, summarized, analyzed and presented using: sigma plot program version 12.5, Microsoft Office Excel (2017). P-values was considered significant when it was equal to or less than 0.05, and are highly significant when P-values<0.01 [31].

RESULTS:

Effect of Liraglutideon neurobehavioral analysis

Compared to the healthy control, rotenone could significantly lower the mean ± SEM value of locomotion duration (seconds) (1638±1) ($p<0.01$), figure (1), also revealed a significantly shorter distance travelled value of mean ± SEM (cm) (36±0.5) ($p=0.007$), figure (2), a significantly higher value of mean ± SEM cataleptic state (seconds) (22±9.76), figure (3), a significantly longer duration to cross the balance beam (seconds) expressed as value of mean ± SEM (40±1), figure (4), and a significantly higher number of slips while crossing the balance beam expressed as value of mean ± SEM (12±1) ($p<0.01$), figure (5). Compared to rotenone induced group, Liraglutide could significantly increase the mean ± SEM locomotion duration (4169±1143), figure (1), reduce the cataleptic state value of mean ± SEM (3±0.05), figure (3), reduce the duration to cross the balance beam value of mean ± SEM (5.9±2.1), figure (4) ($p<0.01$). Compared to pramipexole treated group, Liraglutide could significantly increase the mean ± SEM value of open field locomotion duration (4169±1143), figure (1), the duration to cross the balance beam mean ± SEM value (5.9±2.1), figure (4), and number of slips mean ± SEM value (5.8±1.874), figure (5), and significantly reduce the mean ± SEM value duration to slip down the vertical pole 15.3±10.87 ($p<0.01$), figure (6). No significant difference was found in the rest of tests.

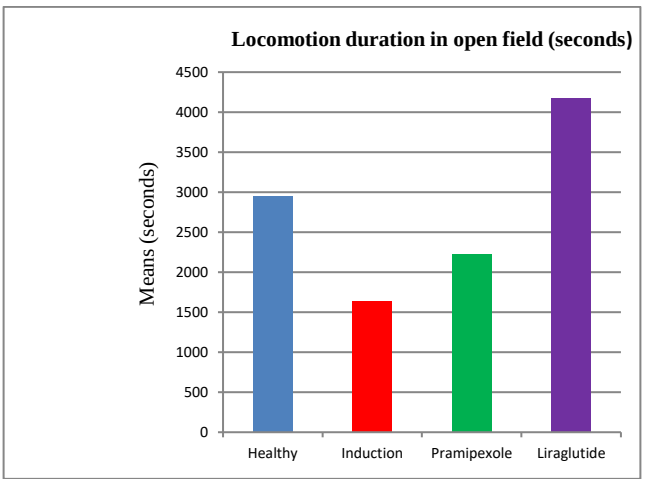


Fig.1: Effects of rotenone, pramipexole, and Liraglutide on locomotion duration in open field test

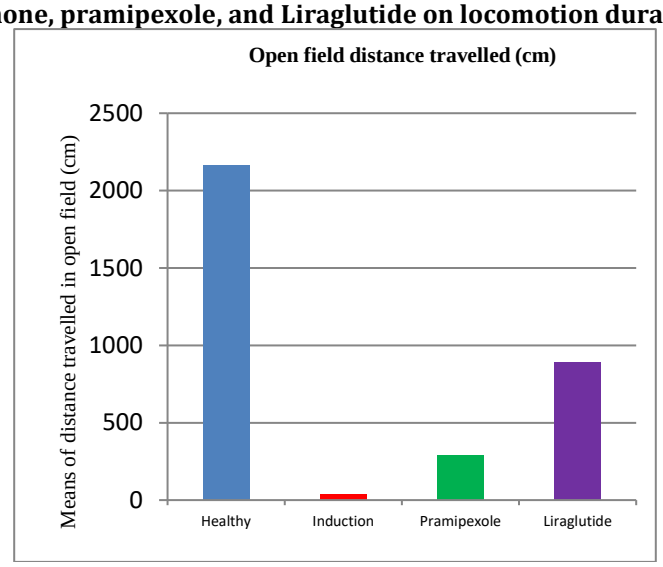


Fig.2: Effects of rotenone, pramipexole, and Liraglutide on distance travelled in open field test

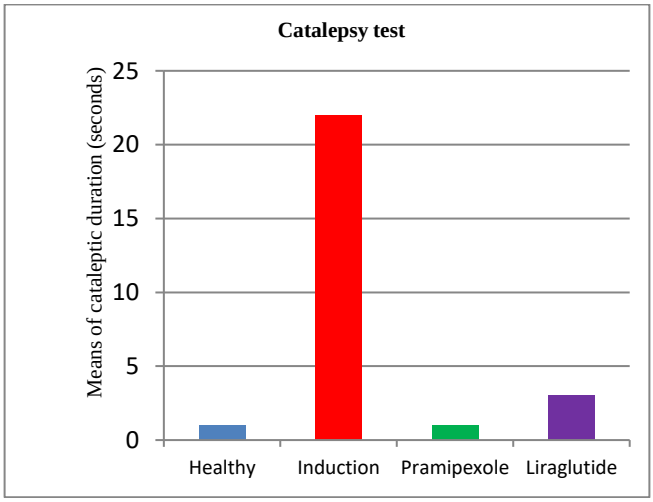


Fig.3: Effects of rotenone, pramipexole, and Liraglutide on locomotion catalepsy test

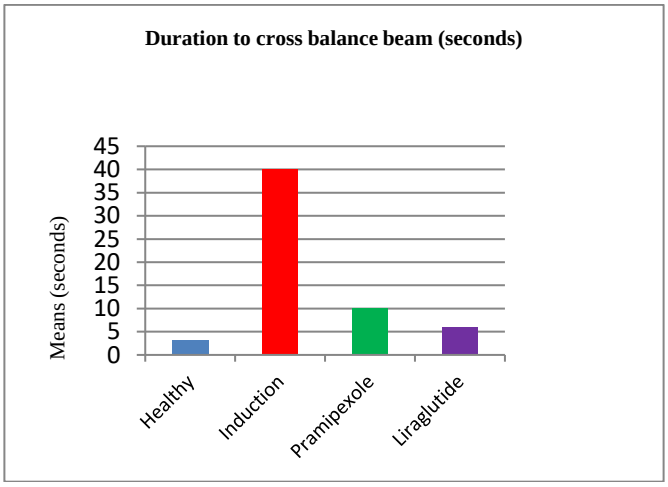


Fig.4: Effects of rotenone, pramipexole, and Liraglutide on duration to cross the balance beam

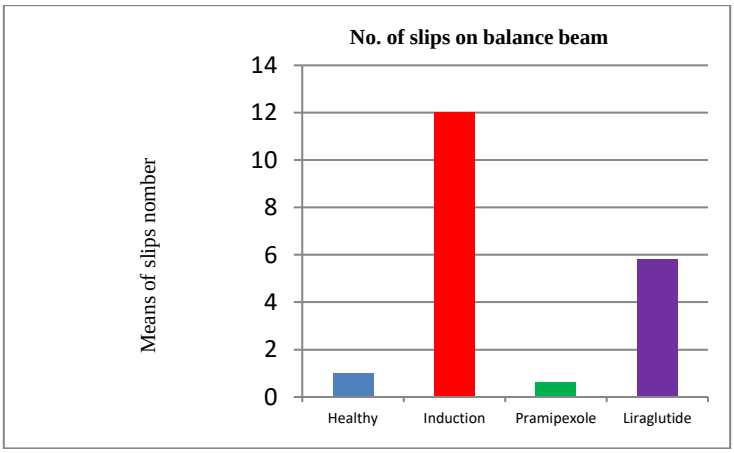


Fig.5: Effects of rotenone, pramipexole, and Liraglutide on slips number on the balance beam

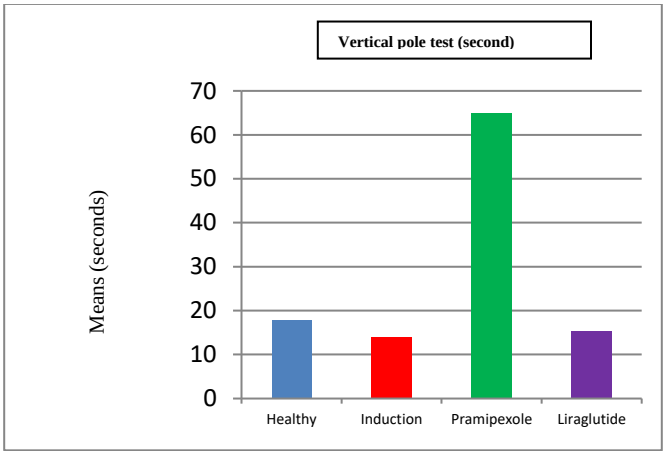


Fig.6: Effects of rotenone, pramipexole, and Liraglutide on vertical pole test

Effect of Liraglutide on Biochemical parameters: Compared to the healthy control, rotenone induced group showed a significantly lower mean ± SEM value of DA tissue concentration (156.315±72.29), figure (7), TH (0.113±0.029), figure (8), and IL-6 (20.104±6.120) ($p<0.01$), figure (10), a significantly higher mean ± SEM value of IL-1β levels (468.109±100.87) ($p<0. 01$) figure (9), cas-3 level (1.312±0.545) ($p=0.01$), figure (11), Cyt-c levels (1.787±1.137) ($p=0.028$), figure (12), and

MPO (0.148±0.125) ($p<0.05$), figure (13). Compared to the rotenone induced group, Liraglutide could significantly increase mean ± SEM value DA content of midbrain tissue samples (1350.281±495.271), figure (7) and significantly reduce that of IL-1β (118.055±72.579), figure (9), cas-3 (0.421±0.161), figure (11), and Cyt –c (0.304±0.131), figure (12), ($p<0.01$). Compared to pramipexole treated group, Liraglutide could a significantly increase mean ± SEM value IL-1β tissue content (118.055±72.579), figure (9). No significant differences were found in the rest of tests.

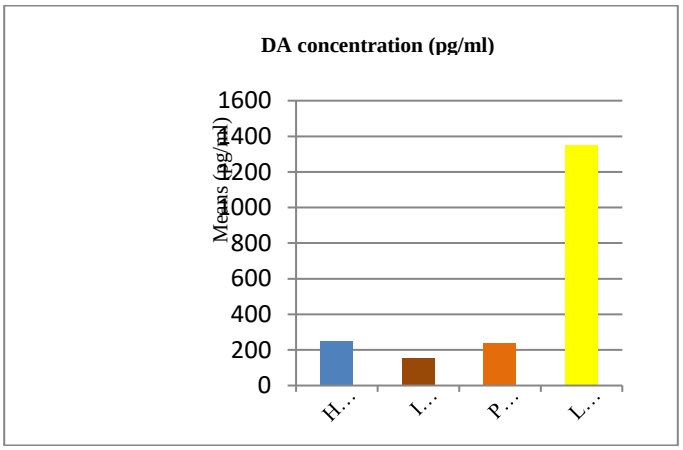


Fig.7: Effects of rotenone, pramipexole, and Liraglutide on DA tissue concentration

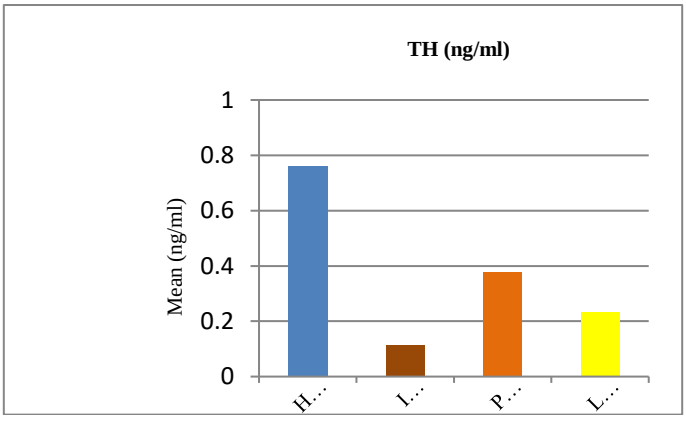


Fig.8: Effects of rotenone, pramipexole, and Liraglutide on TH tissue concentration

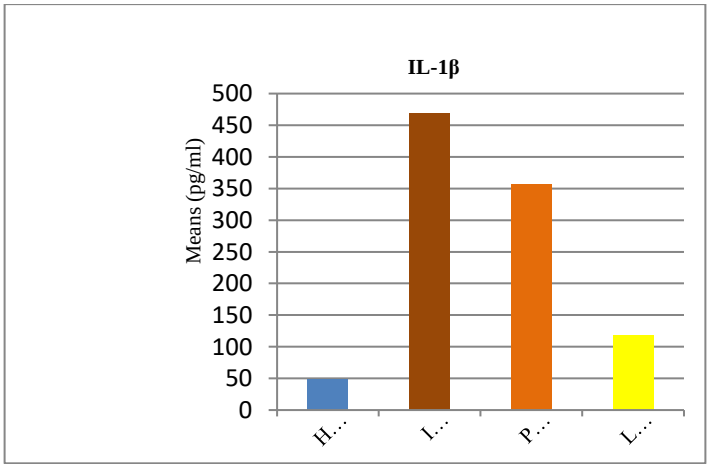


Fig.9: Effects of rotenone, pramipexole, and Liraglutide on IL-1β tissue concentration

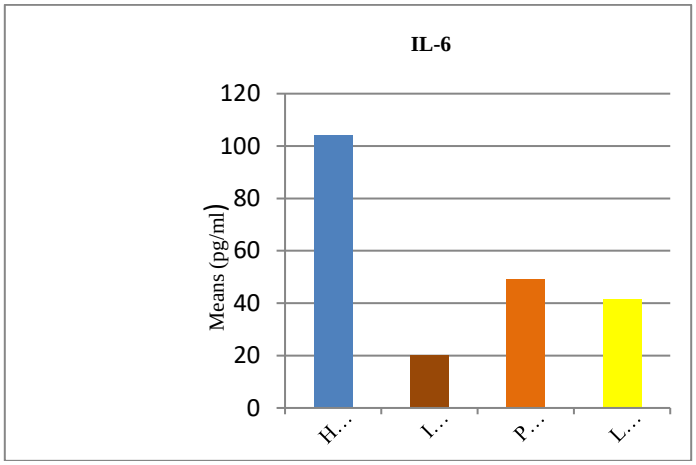


Fig.10: Effects of rotenone, pramipexole, and Liraglutide on IL-6 tissue concentration

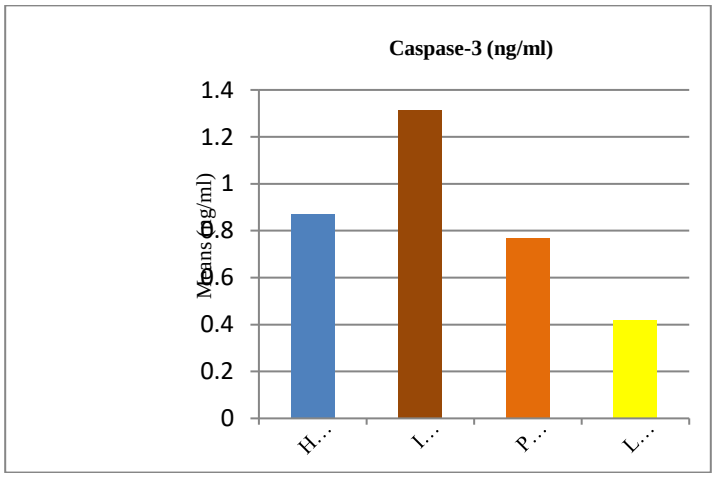


Fig.11: Effects of rotenone, pramipexole, and Liraglutide on Cas-3 tissue concentration

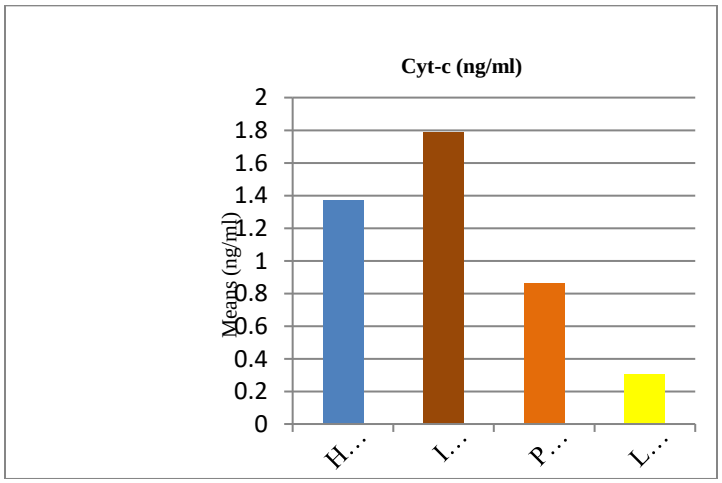


Fig.12: Effects of rotenone, pramipexole, and Liraglutide on Cyt-c tissue concentration

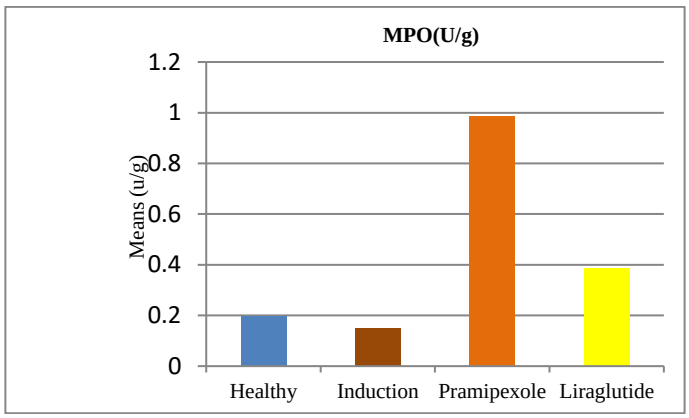


Fig.13: Effects of rotenone, pramipexole, and Liraglutide on MPO tissue concentration

Immunohistochemical analysis of the study groups

Using SNCA (Synuclien alpha) poly clonal antibody the reaction was detected as fine small brownish at the site of the reaction. The pairwise comparisons among the study groups showed a highly significant increase in SNCA expression (H-score) of the rotenone group as compared to the rest of the study groups in terms of (mean ± SD)(5.515±1.950)($p<0.01$). Liraglutide treated group had a significantly higher H-score (3.964±1.402) as compared to pramipexole group (1.512±0.535), ($p<0.01$), for the healthy control the H-score mean value was 1.069±0.378, figure (14).

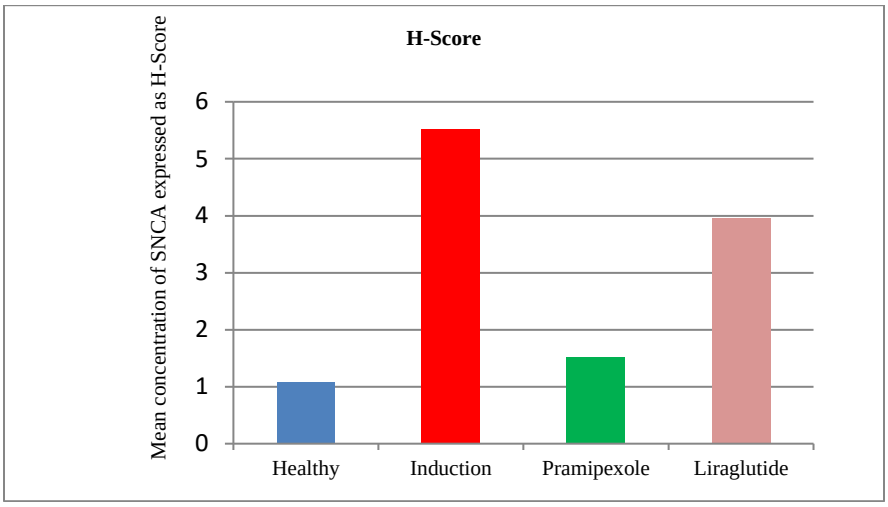
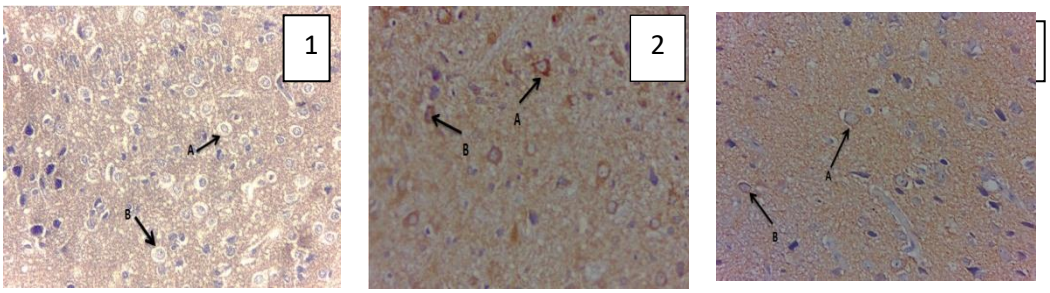


Fig.14: Effects of rotenone, pramipexole, and Liraglutide on SNCA tissue concentration expressed as H-score



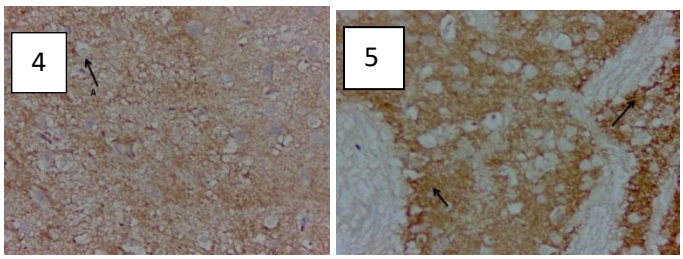


Fig.15: Reaction of SNCA, x40 in (1): healthy control group, A & B is non-stained neuronal cells. (2): negative control group,. A & B are strongly stained cells. (3): positive control group. A&B is moderately stained cells. (5): Arrow refers to a strong and focal expression of SNCA

DISCUSSION

Unfortunately, presently available therapies do not halt PD progression. Biologic disease-related dysfunctions and the pharmacological properties of drugs interact and often induce drug-related complications such as motor and non-motor fluctuations [32]

Effects of Liraglutideon neurobehavioral analysis

Rotenone group showed the least locomotors activity and mobility, among all the other groups which agrees with (33). The significantly longer duration to cross the balance beam of the rotenone group and the highest number of slips, agrees with [34], the significantly higher cataleptic state agrees with [35], the significant lowering of both DA and TH tissue concentration shown un the previous studies had also been found here and was considered as the underlying cause for the neurobehavioral impairment reported in the current study. The pramipexole group had revealed the best motor coordination, by selectively exciting dopamine D3 receptors [36]. Other protective mechanisms may include antioxidant properties, anti- apoptotic activities, and cytotrophic effects [37]. Liraglutide treated group showed the most significant increase in locomotion duration (seconds) as compared to all the other groups, with a significant increase inDA tissue concentration, this agrees with (Ghada et al), catalepsy was reduced by Liraglutide which aggress with Wei Zhen et al who found similar results, Liraglutide improved MPTP-induced motor impairments in mice model of parkinsonism, by rescuing the decrease of tyrosine hydroxylase (TH) levels to protect dopaminergic neurons in the substantianigra and striatum [39], all the previous studies attributed this motor improvement for the fact that Liraglutide preserved (SNpc) and (TH) with prominent increase of the striatal DA content beside the other neuroprotective mechanisms proved in the study (38-40]

Effects of Liraglutideon biochemical parameters

Damage and peripheral inflammation are common triggers for elevated IL-1β found in the current study [41], results revealed by rotenone indicated a neurobehavioral abnormalities concomitant with PD, in addition to increased cas-3 level and might be attributed to AGE-RAGE interaction and the subsequent NFκB activation [42], increased Cyt- c, mainly because of rotenone induction of mitochondrial complex I substrate-supported mitochondrial ROS production, revealed by DNA fragmentation, Cyt-c release, and cas-3 activity [43]. A number of studies had reported that the neuroprotective role of Liraglutide in Parkinson disease is represented by decreasing the level of nigral inflammatory mediators such as: IL1 β, IL-6, and inhibits the adhesion of monocyte and decrease the atherosclerosis in vivo [42], (Ghada et al) also found that Liraglutide activated microglia, gliosis, and other pathological changes [40]. Liraglutide could significantly lower cas-3 levels, this agrees with [45], A reduction of the pro-apoptotic signaling molecule BAX and an increase in the anti-apoptotic signaling molecule Bcl-2 was also reported by (Wei Zhen et al.), after treatment with Liraglutide. The results demonstrate that Liraglutide show promise as a novel treatment of PD. [38]. Liraglutide improved (MPTP)-induced motor impairments in mice model of Parkinsonism by alleviating the inflammation response, inhibited the apoptosis pathway, and also increased autophagy- related protein expression, to protect dopaminergic neurons in the SNc and striatum [39]. Liraglutide seems to promote neuronal survival and attenuate apoptosis and oxidative stress in the brain [46].

Immunohistochemical analysis of the study groups

Rotenone induced an up regulation of alpha-Synuclien protein levels through the stimulation of its de novo synthesis rather than through a

reduction of their chaperone-mediated Autophagy (CMA)-mediated degradation [47]. Pramipexole reduces the relative expression level of SNCA in the tissue samples [48]. Liraglutide could also significantly reduce SNCA, a similar finding had been reported by Liping et al, when Liraglutide improved MPTP-induced motor impairments, rescued the decrease of (TH) levels, reduced the accumulation of SNCA, alleviated the chronic inflammation response in the brain that protects dopaminergic neurons in the SNc and striatum. [49].

CONCLUSION

Liraglutide has anti-inflammatory, and anti-apoptotic properties that are promising to help in PD, may modify L-dopa effect and/or allowing the use of L-dopa with fewer side effects.

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