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The Anti-Parkinson Effects of Cyanara Scoluymus (Artichoke) Extract in Rat Model of Rotenone Induced Parkinsonism

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Abstract
Using the classical dopamine-based anti-Parkinson drugs is still associated with a broad spectrum of dilemmas that created the urgent need to include drugs that targets the other pathological events that accompanies the neurodegeneration process such as: oxidative stress, inflammation and apoptosis, and formation of α-Synuclien aggregates, the phytochemical contained in artichoke and using it as nootropiclead to expect further neuroprotective effects. This study aims to investigate the anti-inflammatory, anti-oxidant, and anti-apoptotic effects of the dry whole artichoke extract. By measurement of dopamine (DA), tyrosine hydroxylase (TH), IL- 1β, IL-6, cytochrome-c (Cyt-c), caspase-3 (Cas-3), myeloperoxidase (MPO) , and α-synuclin (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 of induction), Artichoke group (as in induction+ artichoke 200 mg/kg orally after 30 min of induction). Compared to the induction group, artichoke could significantly increase open field test locomotion duration , improved cataleptic state, balance beam, and vertical pole performance, with significant increase of DA tissue concentration, and IL-6, significantly lower IL-1β, cas-3, cyt- c ($p<0.01$). Compared to pramipexole artichoke group exhibited longer duration to cross the balance beam, higher number of slips, shorter duration to slip down the vertical pole, and significantly lower IL-1β tissue concentration ($p<0.01$). It was concluded that artichoke has potential anti-Parkinson effects that is worthy of further research.
Keywords: Cyanara Scoluymus (Artichoke), Parkinson disease (PD).

Introduction

Parkinson's disease (PD) is an intractable disease resulting in localized neurodegeneration of dopaminergic neurons of the substantianigra pars compacta (SNc) (1). PD is the second most common neurodegenerative disease, afflicting 1% of the population above the age of 65 (2). The prevalence of PD in the U.S. is projected to steadily increase, reaching 2 million by 2030 (3).A wide range of both environmental and genetic risk factors have been implicated in the pathogenesis of PD (4). 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 (5), the target of many therapeutic approaches (6). Apoptosis as a main mechanism of neuronal death in PD is mediated by a number of initiator and executioner caspase (7). Postmortem and *in vitro* studies illustrated elevated activity of cas-3 and increased expression of active cas-3 in SNc. (8). Caspase inhibitors have also been shown to rescue neurons from death in cell models of PD (9). SNCA has been suggested to lead to oxidative stress (OS) and the release of cyt-c into the cytosol, in *vitro* systems. Subsequent to its release into the cytoplasm, cyt-c interacts with pro-survival, antiapoptotic proteins, triggering mitochondria- mediated apoptosis (10). Neuroinflammation plays an important role in the pathogenesis of PD; its resolution is a process that allows for inflamed tissues to return to homeostasis (11). 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 (12).Elevated levels of the proinflammatory interleukins IL-1β, IL-6have also been detected in the striatum (13). 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

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metabolites are involved in the production of reactive oxygen species (ROS)(14). Some other exclusive ways, in which ROS is specifically produced in nigrostriatal DA ergic neurons, includes mitochondrial impairment, reactive iron stored at neuromelanin (NM) and inflammation (15). Cyanara Scoluymus is a pharmacologically important medicinal plant containing phenolic acids and flavonoids, belongs to the family of Asteraceae (Compositae) (16), it is to be investigated here for possible anti-Parkinson effects.

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 (17), in a dark room, every 48 hr for 20 days, Gr III: The pramipexole group treated with oral pramipexole solution 1 mg/kg (18). Gr VI: Artichoke extract 200 mg/kg (19), both of pramipexole and artichoke extract were given every 48 hr by gastric gavage, 30 minutes after rotenone injection for 20 days.

Preparation of drugs

Pramipexole commercially available as oral tablets (Sifrol®), Artichoke leaves were extracted in ethanol by soxhlet apparatus, then the dry extract was used; both were given orally by gastric gavage.

Induction method

The pesticide rotenone has been shown to cause systemic inhibition of mitochondrial complex I activity, with consequent degeneration of DA neurons along the nigrostriatal pathway, as observed in PD (20) which can cause OS and lead to selective degeneration of striatal-nigral DA neurons. Besides, aggregation of SNCA and polyubiquitin, activation of Astrocytes and microglial cells, inflammatory reaction, and neuronal apoptosis are all involved in the mechanisms of rotenone-evoked Parkinsonism (21). Rotenone powder was dissolved in Dimethyl sulfoxide (DMSO) to get a (0.00761 Mm solution) (17), 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 (22), the solution was kept refrigerated in dark glass container and wrapped with Aluminum foil, since rotenone is susceptible to rapid photodegredation (23).

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 was preserved in 10% formalin solution for 48 hr then paraffin sections were prepared according to (24) as follows: fixation, dehydration, clearing, impregnation, embedding, sectioning, dewaxing, 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 (25), Balance beam test (26), Catalepsy test (28), and Vertical pole test (28).

Biomarkers' measurement

Based on ELISA principle, tissue concentrations of DA, TH, IL-1β, IL-6, cas-3, and cyt-c were measured. MPO was measured based on a photochemical reaction using a spectrophotometer, all were executed according to the manufacturer's instructions.

Immunohistochemical study (IHC)

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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).Histoscore 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 Histoscore with a potential range of 0–300 was calculated as follows: Histoscore (H-score)=((1 × % weakly stained cells)+(2 × % moderately stained cells)+(3 × % strongly stained cells))(29).

Preliminary qualitative phytochemical analysis of artichoke dry extract

Chemical tests were carried out on artichoke ethanolic extract using standard procedures to identify the bioactive groups in it (30), to test for the presence or absence for steroids, alkaloids, flavonoids, Anthraquinones, cardiac glycosides, and saponins. This was followed by the identification of the major flavonoids contained in the extract by HPLC.

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 if ≤0.05, and are highly significant when P-values<0.01(31).

Results:

Effects of Artichoke on neurobehavioral analysis:

Compared to the healthy control, rotenone could significantly lower the mean ± SEM of locomotion duration (seconds)(1638±1) ($p<0.01$) (fig 3-1), also revealed a significantly shorter distance travelled value of mean ± SEM (cm) (36±0.5)($p=0.007$) (fig 3-2), a significantly higher value of mean ± SEM cataleptic state (seconds) (22±9.76) (fig 3-3), a significantly longer duration to cross the balance beam (seconds) expressed as value of mean ± SEM (40±1) (fig 3-4), and a significantly higher number of slips while crossing the balance beam expressed as value of mean ± SEM(12±1) ($p<0.01$),(fig 3-5). Compared to rotenone induced group, artichoke could significantly increase locomotion duration (seconds) expressed as value of mean ± SEM (2588±370.154) in open field (fig 3-1), a significantly lower cataleptic state (2.1±0.876) (fig 3-3), a significantly shorter duration to cross the balance beam (14.6±3.2) (fig 3-4), a significantly lower number of slips (4.1±1.853) (fig 3-5), a significantly longer duration to slip down the vertical pole (40.3±20.688) ($p<0.01$), (fig 3-6). Compared to the pramipexole treated group the artichoke group could significantly show a longer duration to cross the balance beam as expressed in mean ± SEM (14.6±3.2) ($p<0.01$) (fig 3-1), a significantly higher mean ± SEM value of number of slips (4.1±1.853) ($p<0.01$) (fig 3-5), a significantly shorter duration to slip down the vertical pole in terms of mean ± SEM (40.3±20.688). ($p<0.01$), (fig 3-6).

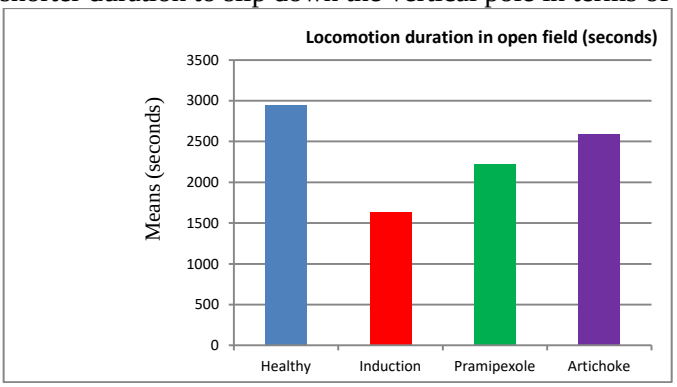

Figure (3-1): Effects of rotenone, pramipexole, and artichoke on locomotion duration in open field test

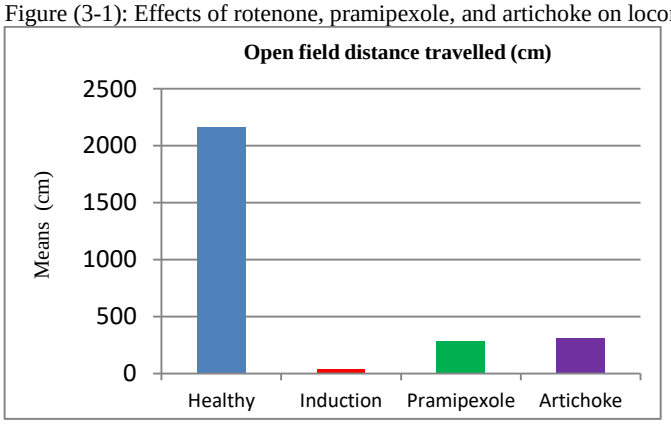

Figure (3-2): Effects of rotenone, pramipexole, and artichoke on distance travelled in open field test

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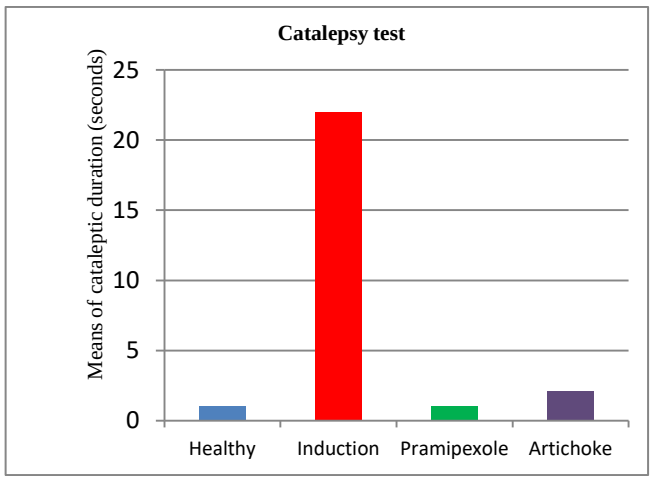

Figure (3-3): Effects of rotenone, pramipexole, and artichoke on catalepsy test

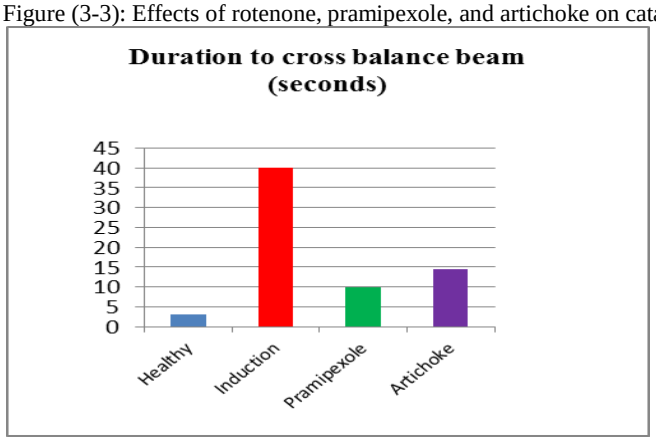

Figure (3-4): Effects of rotenone, pramipexole, and artichoke on balance beam

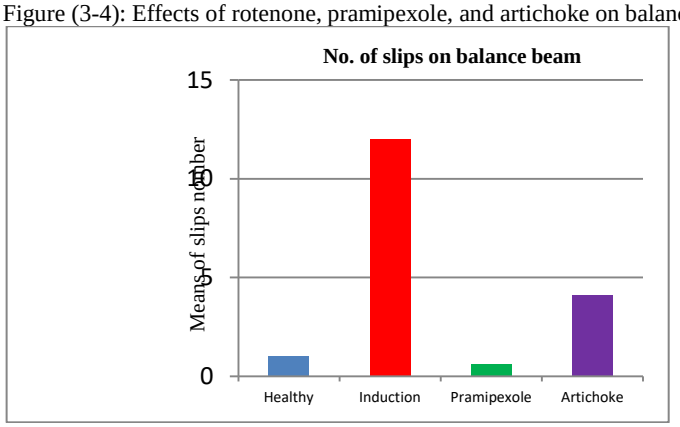

Figure (3-5): Effects of rotenone, pramipexole, and artichoke on slips number on balance beam

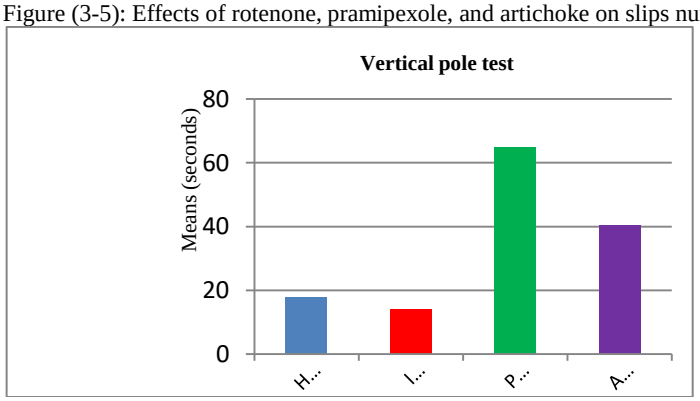

Figure (3-6): Effects of rotenone, pramipexole, and artichoke on vertical pole test

Effects of Artichoke 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) (fig 3-7), TH (0.113±0.029) (fig 3-8), and IL-6 (20.104±6.120) ($p<0.01$) (fig 3-10), a significantly higher mean ± SEM value of IL-1β levels (468.109±100.87)($p<0.01$) (fig

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3-9), cas-3 level(1.312±0.545) (p=0.01)(fig 3-11), cyt-c levels (1.787±1.137) (p=0.028) fig (3-12), and MPO (0.148±0.125), (p<0.05)(fig 3-13).Compared to the rotenone induced group, artichoke showed a significantly higher in mean ± SEM value of DA tissue concentration (193.45±52.423) (P<0.01) (fig 3-7), IL-6 (444.431±9.225)(p=0.021)(fig 3-10) , a significantly lower mean ± SEM value of IL-1β levels (77.746±18.707)(p<0.01) (fig 3-9), cas-3 level (0.61±0.193)(p<0.01) (fig 3-11), and cyt-c (0.405±0.296)(p=0.001)(fig 3-12).

Compared to the positive control group the artichoke group showed a significantly lower mean ± SEM value of IL-1β levels (77.746±18.707)(p<0.01)(fig 3-9).

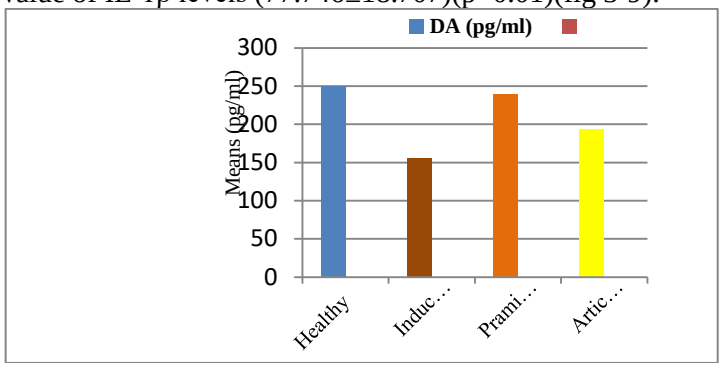

Figure (3-7): Effects of rotenone, pramipexole, and artichoke on tissue concentration of DA(pg/ml)

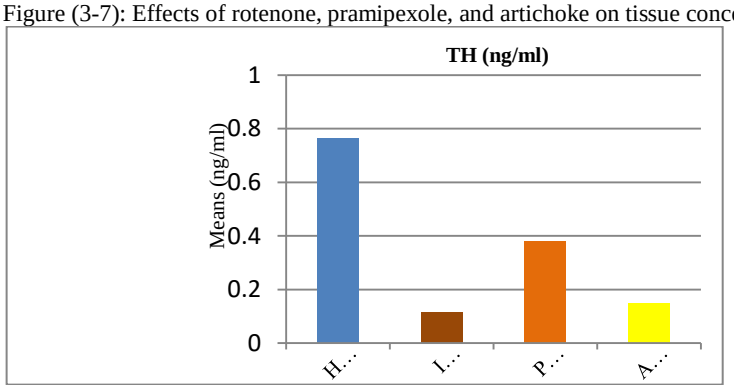

Figure (3-8): Effects of rotenone, pramipexole, and artichoke on tissue concentration of TH(pg/ml)

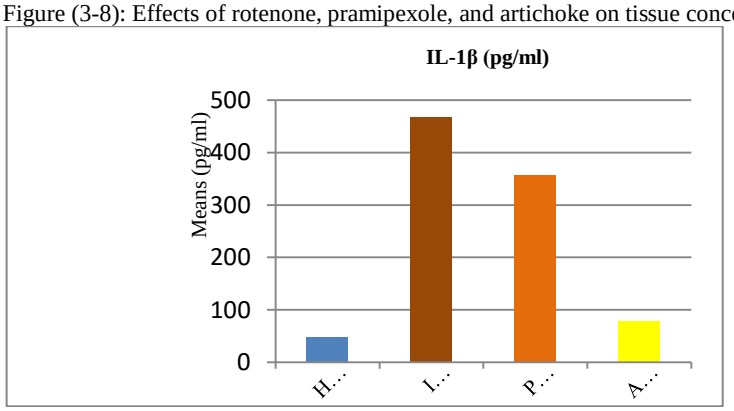

Figure (3-9): Effects of rotenone, pramipexole, and artichoke on tissue concentration of IL-1β(pg/ml)

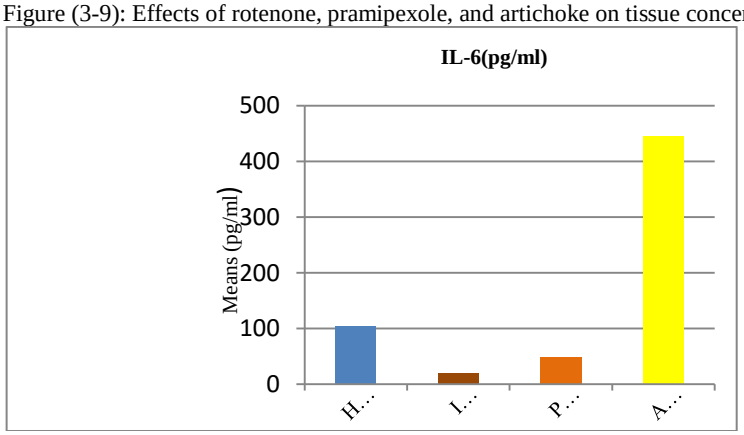

Figure (3-10): Effects of rotenone, pramipexole, and artichoke on tissue concentration of IL-6 (pg/ml)

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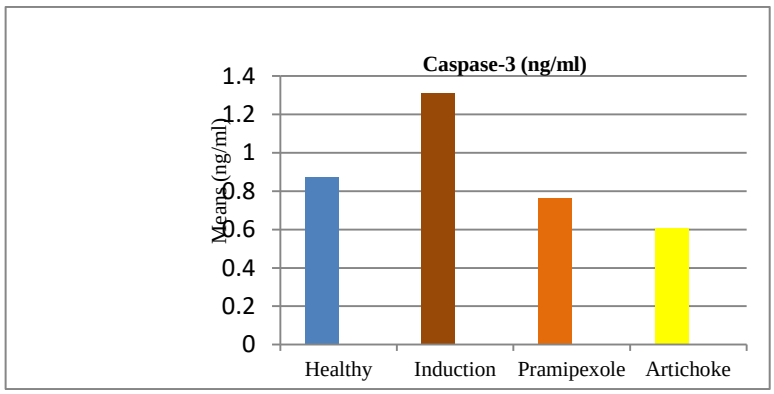

Figure (3-11): Effects of rotenone, pramipexole, and artichoke on tissue concentration of Cas-3(ng/ml)

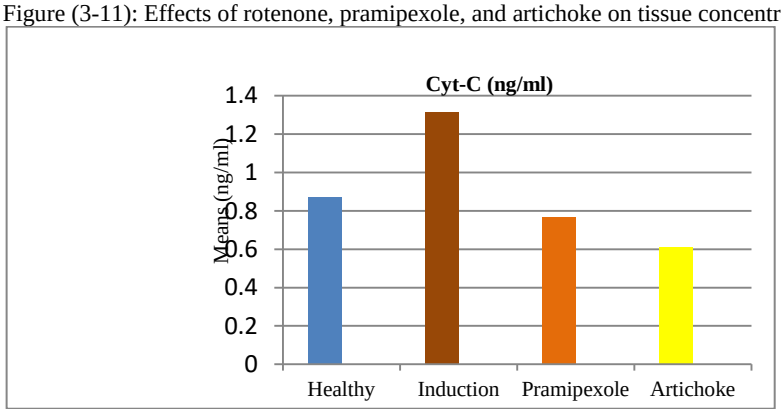

Figure (3-12): Effects of rotenone, pramipexole, and artichoke on tissue concentration of Cyt-c (ng/ml)

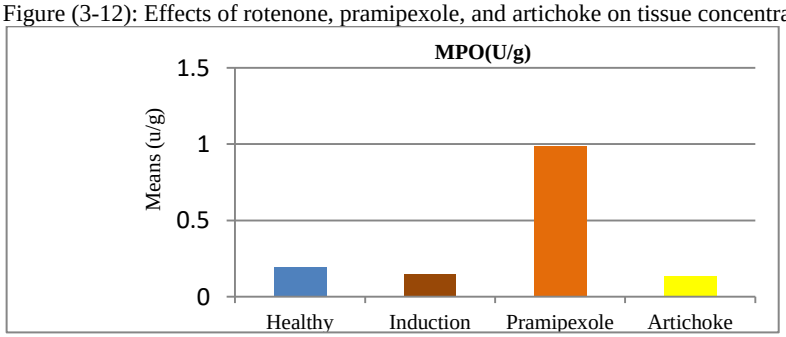

Figure (3-13): Effects of rotenone, pramipexole, and artichoke on tissue concentration of MPO (u/g)

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 pair wise comparisons among the study groups showed a highly significant increase in SNCA expression (H-score) of the rotenine group as compared to the rest of the study groups in terms of (mean±SD)(5.515±1.950)($p<0.01$), for pramipexole the score mean was(1.512±0.535), for the healthy control the H-score mean value was 1.069±0.378, while for artichoke group it was (1.438±0.508). (fig 3-14).

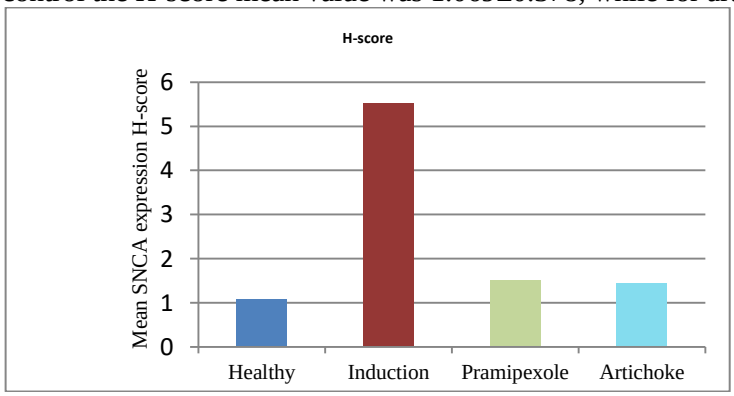

Figure (3-14): Effects of rotenone, pramipexole, and artichoke on SNCA tissue concentration expressed as H-score

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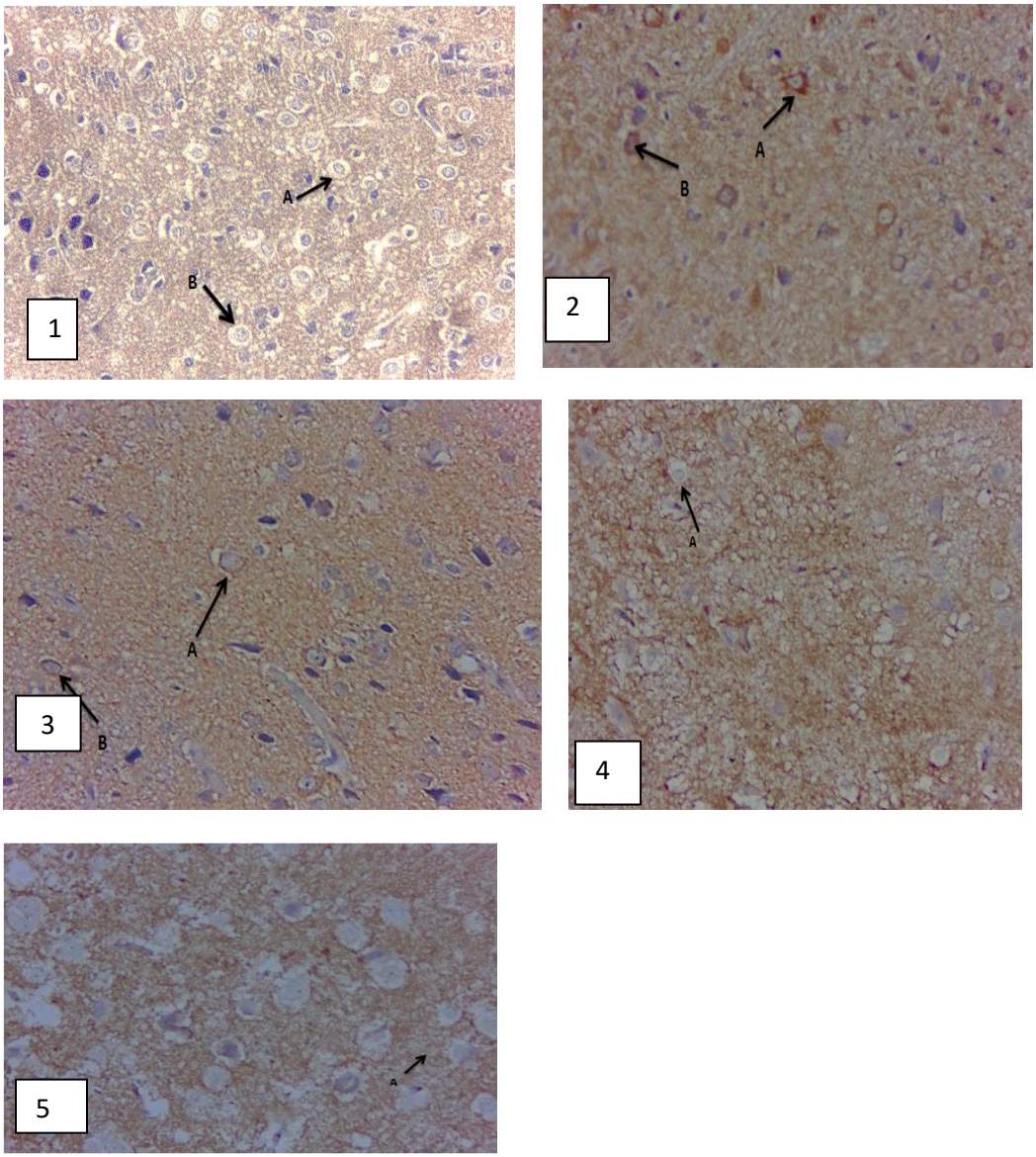

Figure (3- 15): Reaction of SNCA, x40 in (1): healthy control group, A & B are non-stained neuronal cells. (2): rotenone group,. A & B are strongly stained cells. (3): pramipexole group. A& B are moderately stained cells. (5): Artichoke treated group. A is a non-stained cell

Phytochemical analysis of artichoke extract

The results qualitative phytochemical screening of the dry artichoke extract revealed the presence of alkaloids, flavonoids, steroids. By comparing the obtained retention times with the ones of standards based on HPLC. The flavonoids that were identified in artichoke extract were: Rutin, Caffeic acid, Quercetin, Kaempferol, Myricetin, and Catechin. See table (3-1)

Table (3-1): HPLC analysis of the major flavonoids in Artichoke dry extract shows retention time in minutes

Standards	Retention time	Standards	Retention time	Standards	Retention time
Rutin	2.573	Quercetin	6.52	Myricetin	8.133
Caffeic acid	4.10	Kaempferol	7.320	Catechin	11.90

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 nonmotor fluctuations (32).

Effects of Artichoke on 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 in the previous studies had also been found here and was considered as the underlying cause for the neurobehavioral impairment reported in the current

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study. Artichoke could improve most of the behavioral defects induced by rotenone, this shows the efficiency of the active artichoke constituents such as phenolic compounds (36,37), alkaloids (38; 39, 40), , flavonoids (41), steroids (42), catechins (43),Quercetin (44, 45), Rutin (vitamin p) (46), Myercetin(47), Kaempferol (48), Caffeic acid (49). The pramipexole group had revealed the best motor coordination, by selectively exciting D3 receptors (50). Other protective mechanism s may include antioxidant properties, anti- apoptotic activities, and cytotrophic effects (51). The artichoke was not more efficient than pramipexole as anti-Parkinson drug according to the results reported in this study.

Effects of Artichoke on biochemical parameters

Damage and peripheral inflammation are common triggers for elevated IL- 1β found in the current study (Laye et al., 2000), results revealed by rotenone indicated a Parkinson model as proved by the neurobehavioral analysis, in addition to increased cas-3 level and might be attributed to AGE-RAGE interaction and the subsequent NFκB activation(53), 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 (54). A non-significant effect on MPO levels was revealed, MPO is reported to have dual functionality under rotenone-exposed conditions, and may play a critical regulatory role in modulating pathological and protective events in the brain (55). A larger sample size may be needed for a better outcome. Phenolic compounds in artichoke exhibit neuroprotection through a diversity of important mechanisms: such as management the expression of antiapoptotic factors, promote different signaling pathways in the cell prevent protein aggregation, and regulate the major proteins degradation pathways (36,37). Neuroprotective activity of alkaloids was also studied by (56), through capturing free radicals, or through binding to catalysts involved in different oxidation processes occurring within the human body. (40). The possible anti-inflammatory, and anti-apoptotic mechanisms of alkaloid action in experimental animals are diverse and multiple as mention by (44), and may involve the brain's enzyme and receptor systems, with significant protective effect against neurodegeneration associated with PD(57), the anti-inflammatory, activities of steroids in many planet extracts was studied (42, 58), the Chemokines, produces recruitment of lymphocytes leading to tissue damage that are expressed during the inflammatory process are inhibited by steroid (58). Catechins can modulate the cellular and molecular mechanisms through the inflammation-related NF-κB and the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathways. (43), Suppression of the increased cytokines and inflammatory proteins (59). Catechins suppress microglia activation by decreasing the expression of inducible nitric oxide synthase (iNOS) giving an anti-neuroinflammatory impact on microglia, steroids also show an anti-inflammatory effect (60), their neuroprotective activity acts through suppression of apoptosis as an example (61). Quercetin mainly affects the enzyme systems involved in the generation of inflammatory processes. (44), reducing lipid peroxidation and inflammatory cascade pathways inhibition, Quercetin also exert neuroprotection (45). Rutin (vitamin p) has been shown to have anti-inflammatory actions, improved locomotors activity and motor coordination, DA content, increase dopaminergic D2 receptors in striatum (46), it also attenuates the expression of Cas-3gene expression, upregulate the protective genes, including Th. (41). Myercetin can decrease Cas-3 expression (62) , Kaempferol was reported to suppress secretion IL-1β and IL-6 with evidence of reduced (MPO) activity (48), it also could inhibit the formation of SNCA and destabilize the performed one (63) possibly by no covalent binding of the inhibitory flavonoids to alpha-synuclin led to the restriction of the conformational changes in this natively unfolded protein and to the stabilization of soluble flavonoid-modified species of alpha-synuclin (monomers and oligomers) (64). Caffeic acid amended most of rotenone-induced motor deficits, lessened microglia expression and inflammatory mediators and improved the nigral TH immunostaining and confirmed the anti-inflammatory activity of Caffeic acid and highlighted its neuroprotective activity against rotenone-induced neurodegeneration in mice. (49)

Immunohistochemical analysis of the study groups

Rotenone induced an up regulation of alpha-synuclin protein levels through the stimulation of its de novo synthesis rather than through a reduction of their chaperone-mediated Autophagy (CMA)-mediated degradation (65). Pramipexole reduces the relative expression level of SNCA in the tissue samples (66). Flavonoids significantly affected SNCA propensity to aggregate in vitro through binding and stabilizing the certain protein conformations.(67), Catechins, are neuroprotective polyphenolic compounds (68), Presence of Quercetin disaggregates α-synuclin fibrils (69).Alkaloids constitute positive roles as an inhibitor of α-synuclin aggregation (70).

Conclusion

Artichoke had revealed beneficial effects against Parkinson disease owing to its anti-inflammatory, and anti-apoptotic properties beside its ability to reduce alpha Synuclien expression, the major constituent of lewy bodies, and the Pathological hallmark of PD.

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