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Anti-Parkinson's Activity of *Uncaria rhynchophylla* Extract on Rotenone Induced Parkinsonism Model in Male Albino Rats

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***Abstract*:** *Present study investigated the curative effects of Uncaria rhynchophylla aqueous extract (URE) on Parkinson's disease. Fifty male albino rats were equally divided into 5 groups. Group I, gavaged oral saline for 35 days and injected saline subcutaneous (S.c) at 48 h interval for the first 20 days. Group II, gavaged URE 500 mg/kg daily for 35 days and injected saline S.c at 48 h interval for the first 20 days. Group III, gavaged saline daily for 35 days and injected DMSO/saline 20% (v/v) S.c at 48h interval for 20 days. Group IV, gavaged saline daily for 35 days and injected rotenone (1.5 mg/kg/ 48h) in DMSO/saline 20% (v/v) S.c for 20 days. Group V, received S.c injection of rotenone (1.5 mg/kg/ 48h) in DMSO/saline 20% (v/v) interval for 20 days and gavaged URE 500 mg/kg daily for the left 15 days. Brain homogenate dopamine (DA), tyrosine hydroxylase (TH), tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), Total oxidant status (TOS), nitric oxide (NO) total antioxidant capacity (TAC) and reduced glutathion (GSH) were estimated. URE exerts anti-Parkinson's activity via increasing DA, TH, TAC and GSH as well as reducing TNF-α, IL1-β, IL-6, TOS and NO. URE possesses neurotherapeutic properties against Parkinson's disease.*

Keywords: *Parkinson's disease, brain, oxidative stress, Uncaria.*

1. Introduction

Parkinson's disease (PD) is a major age-related neurodegenerative disorder with cardinal features of bradykinesia, postural abnormalities, rigidity, and resting tremors [1]. The neuropathological hallmarks of PD are the loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNc) and the formation of intra-neuronal proteinaceous inclusions, called Lewy Bodies (LBs) [2]. The motor impairments in PD manifest only when there is a reduction of 60–80% of dopaminergic neurons [3]. The loss of dopaminergic neurons leads to the reduction of striatal dopamine (DA) levels. As a consequence, tyrosine hydroxylase (TH), the rate-limiting step in the biosynthesis of DA, can be involved in the pathogenesis of PD at several different levels [4].

Several animal models of PD exist, each with its own advantages and disadvantages. Rotenone, the ideal model, is a naturally occurring insecticide and a specific inhibitor of mitochondrial complex I that easily crosses the blood-brain barrier. Systemic administration of rotenone in low doses (1.5–2.5 mg/kg) has the ability to create a chronic progressive animal model of PD [5] by specific degeneration of the nigrostriatal pathway similar to that observed in human PD [6]. Recently, it was reported that rotenone model is highly reproducible and may be more relevant to test new neuroprotective strategies [7]. Some studies approved that chronic systemic exposure of rotenone to rats causes many features of PD, including nigral degeneration, a decrease of striatal dopamine levels, and motor dysfunction [8-10] as well as α-synuclein aggregation [11-13].

Pathogenic mechanisms have been implicated in PD including environmental toxins, oxidative stress, mitochondrial dysfunction and apoptosis. Neuronal loss in PD is associated with chronic inflammation, which is

controlled primarily by microglia, the resident innate immune cells to repair tissue after neuronal injury [14]. Activated microglia releases free radicals such as nitric oxide and superoxide, as well as pro-inflammatory cytokines including interleukin 1 beta (IL-1β) and tumor necrosis factor α (TNF-α), and proteases driving progressive neuron damage [15]. In addition, the neurotransmitter DA itself can be a source of oxidative stress, autoxidation of DA generates ROS and quinone. DA quinone has been shown to cause inactivation of the DA transporter and tyrosine hydroxylase [16], and leads to mitochondrial dysfunction [17]. Thus oxidative stress has been hypothesized to be linked to both the initiation and the progression of PD.

Antioxidant remedies, especially the herbal antioxidants, have been extensively used as new perspectives of therapy and research as a therapeutic approach for PD [18]. Uncaria rhynchophylla (UR) is a genus of flowering plants in the family Rubiaceae. It is also known as Cat's Claw. It has about 40 species. Their distribution is pantropical, with most species native to tropical Asia [19]. It contains oxindole alkaloids such as Uncarine, Pteropodine, Isomitraphylline, speciophylline and metraphylline and flavanols like catechin, epigallocatechin, epicatechin and epigallocatechin gallate [20]. Cat's Claw herb, is used around the world for treating immune disorders, gastritis, ulcers, cancer and neuronal protection [21]. Moreover, UR has also been found to inhibit TNF-α and nitric oxide (NO) production in mice in vitro [22].

The goal of this study was to evaluate the neurotherapeutic effect of aqueous extract of UR on rotenone induced parkinsonism in male rats through assessments of DA, TH as well as proinflammatory factors, oxidative stress biomarkers.

2. Materials and Methods

2.1 Drugs and Chemicals

Rotenone and Dimethyl sulfoxide (DMSO) were procured from Wako Pure Chemical Industries, Ltd. Japan. UR (Japan Pharma Co., Japan) that contained 3% alkaloids and 3.5% flavonoids as determined by HPLC. All the chemicals used were of analytical grade.

2.2 Experimental animals

Male albino rats, weighing 150-200g were purchased from VACSERA, Helwan, Egypt, two weeks before the experiment and allowed to climate. Experimental rats were housed in plastic cages at room temperature (25 ± 2 °C). They were kept under natural day light-dark cycle with free access to ad libitum food and water.

2.3 Experimental Design

The experimental animals will be randomly divided into 5 groups that each one contains 10 rats:

Group I (Control -ve): they received saline 0.9 % NaCl daily by oral gavage for 35 days and injected saline subcutaneous (S.c) at 48 h interval for the first 20 days.

Group II (URE): they received UR aqueous extract (URE) 500 mg/kg daily by oral gavage for 35 days and injected saline S.c at 48 h interval for the first 20 days.

Group III (vehicle): they received saline 0.9 % NaCl daily by oral gavage for 35 days and injected DMSO/saline 20% (v/v) S.c at 48 h interval for 20 days.

Group IV (control +ve): they received saline 0.9 % NaCl daily by oral gavage for 35 days and injected rotenone in DMSO/saline 20% (v/v) S.c in a dose 1.5 mg/kg/ 48h for 20 days.

Group V (Treated): they received rotenone in DMSO/saline 20% (v/v) by S.c injection 1.5 mg/kg/48 h interval for 20 days and gavaged URE 500 mg/kg daily for 15 days post retenone injection.

2.4 Induction of rotenone-parkinsonism rat model

Parkinsonism model was induced by subcutaneous injection of rotenone 1.5 mg/kg [23]. Rotenone solution was freshly prepared by dissolving the dose of rotenone in (DMSO/ Saline, 20 % v/v) and injected, in groups IV and V, S.c at 48h interval for the first 20 days of experiment.

2.5 Tissue sampling

At the end of experimental period, animals were deeply anesthetized by diethyl ether. The animals were scarified and their brains were quickly removed and washed with ice-cold saline. The striata of one hemisphere of each brain were isolated and stored at -20℃ for homogenization. Homogenization was carried out as 20% (v/v) in phosphate-buffered saline (0.01 M, pH = 7.4) using a teflon homogenizer. The homogenate was sonicated and centrifuged at 2000×g for 10 min. The supernatants were kept at - 80ºC until performing Enzyme

2.6 Enzyme-linked immunosorbent assay (ELISA)

TNF-α, IL-1β and, IL-6 as cytokines were measured in brain homogenate using specific ELISA kits (Ray Biotech Inc., USA). Moreover, the concentrations of dopamine (CCC Inc, USA), Tyrosin hydroxylase (Cusabio Biotech, Ltd, USA) and total antioxidant capacity (TAC) (Zen-Bio Inc, USA) were determined. All these parameters were measured by ELISA according to manufacturer protocol.

2.7 Total oxidant status (TOS)

TOS was determined using a novel automated measurement method, developed by Erel, [24]. This method depends on oxidation of ferrous molecule into ferric one with enhancement of reaction with glycerol as substrate.

2.8 Reduced glutathione (GSH) and nitric oxide

The Reduced Glutathione was calorimetrically estimated using commercial kits (Oxford Biomedical Research Inc, USA). Nitric oxide measured as the nitrite was determined using Griess reagent, according to the method devised by Griess [25], which is based on measurement of endogenous nitrite concentration as an indicator of nitric oxide production.

2.9 Statistical analysis

Statistics were calculated with SPSS program version 17.0, the means value obtained in the different groups were compared by one way analysis of variance, ANOVA, followed by Duncan's test. All the results were expressed as mean values ± SE and significance was defined as $P< 0.05$.

3. Results

Injection of rotenone resulted in a significant decrease in DA and TH levels ($P<0.05$) by 77% and 63% in striatum brain region as compared to control (-ve). Furthermore, URE administration significantly ($P<0.05$) increased DA by 30% and TH by 37% in group (V) versus control (-ve), (Table 1).

Following injection of rotenone, there was a significant ($P<0.05$) increase in TNF-α IL-1β, and IL-6 by (75%,72%,78%) as compared to control (-ve) group. A significant ($P<0.05$) decrease in TNF-α IL-1β, and IL-6 concentration was observed versus rotenone treated animals, after URE doses of 500 mg/kg in group V (Table 1).

In rotenone-treated rats, there was a significant increase ($P<0.05$) in NO and TOS levels by 73% and 80%, whereas a significant ($P<0.05$) decrease in TAC and GSH were observed compared to control group (77% and 75 %). Following the treatment with URE 500 mg/kg, NO and TOS levels were significantly ($P<0.05$) decreased in group V, while TAC and GSH levels were significantly ($P<0.05$) increased in groups V versus rotenone (control +ve) group (Table 2).

TABLE I: Effect of URE on DA, TH, TNF-α, IL-1β and IL-6 in striatal tissue of rotenone-treated male rats

	DA ng/g	**TH ng/g**	**TNF – α pg/g**	**IL-1β pg/g**	**IL-6 pg/g**
Group I	19.2± 3.5	3.7 ± 0.33	6.7 ± 0.33	8.7± 0.33	5.7± 0.33
Group II	30.8± 0.58[b]	5.3 ± 0.33[a,b]	2.7 ± 0.33[a,b]	2.5± 2.50[a,b]	2.3 ± 0.33[a,b]
Group III	21.7± 0.58 [b]	3.3 ± 0.33 [b]	8.3 ± 0.33[b]	10.3 ± 0.33 [b]	7.3 ± 0.33[b]
Group IV	4.4± 0.50[a]	1.0 ± 0.00 [a]	27.0 ± 2.10[a]	30.7 ± 1.70 [a]	26.7 ± 1.7[a]
Group V	13.6± 1.73[a,b]	2.3± 0.33[a,b]	14.7 ± 0.33[a,b]	19± 1.0[a,b]	16±1.1[a,b]

Values are expressed as mean ± SE (n=10/group). [a]P<0.05 significant as compared to control (-ve) group; [b]P<0.05 significant as compared to control (+ve) group using Duncan test.

TABLE II: Effect of URE on TOS, TAC, GSH and NO in striatal tissue of rotenone-treated male rats

	TOS µmol H2/ O2 equivalent/g	**TAC µm/g**	**GSH µmol/g**	**NO µmol/g**
Group I	6.7 ± 0.88	28.7± 1.7	4.0 ± 0.00	7.7± 0.33
Group II	3.3 ± 0.33[a,b]	45.7 ± 3.2[a,b]	6.0 ± 0.00[a,b]	3.7± 0.33[a,b]
Group III	7.3 ± 0.33 [b]	24.3 ± 0.88 [b]	3.3 ± 0.33 [b]	9.3 ± 0.33 [b]
Group IV	33.3± 1.7 [a]	6.7± 0.33 [a]	1.0 ± 0.00[a]	29.0 ± 1.5 [a]
Group V	12.0± 0.58[a,b]	18.7± 1.3[a,b]	2.7 ± 0.33 [a,b]	13.3 ± 0.33[a,b]

Values are expressed as mean ± SE (n=10/group). [a]P<0.05 significant as compared to control (-ve) group; [b]P<0.05 significant as compared to control (+ve) group using Duncan test.

4. Discussion:

Rotenone is considered one of the best strategies to induce PD in experimental animals by inhibition of mitochondrial complex I as well as its easy access to blood brain barrier [26]. Efforts collaborate to find bet safe strategies to treat PD. The usage of herbal medicine to counteract such problem is a safe alternate to pharmacological drugs [27]. URE aqueous extract was investigated in the present study as anti-Parkinson's agent.

The current study demonstrated that rotenone injection to rats resulted in a significant (P<0.05) decrease in striatal DA and TH levels than control (-ve). These results were in agreement with Alam and Schmidt [28] and Zaitone et al. [23]. They attributed the deficiency of DA to the deficiency of ATP, due to inhibition of mitochondrial complex 1, that keeps ionic gradience of Na+ and K+ suitable for dopamine transmembrane transport or DA uptake to protect neurons. In the current study, TH reduction and subsequent DA reduction were observed in rotenone injected group. This may be attributed to the negative effect of alpha-synuclein in PD [29]. This enzyme is a rate-limiting step in the biosynthesis of catecholamines that catalyzes the conversion of tyrosine to L-DOPA, the precursor of dopamine, which is in turn converted into noradrenalin by dopamine-β-hydroxylase [30]. The loss in TH activity and expression is thought to contribute to the L-DOPA deficiency observed in parkinson's disease [31]. The administration of URE into Parkinsonism induced rats produced an increase of DA and TH levels in group V compared to control group (-ve) at P < 0.05. These results could be attributed to the alkaloids contents of the extract such as rhynchophylline and isorhynchophylline as well as flavonoids contents like catechin and epicatechin. Rhynchophylline has the ability to inhibit serotonin release that promotes dopamine levels in the brain [32, 33]. Moreover, Catechin can inhibit monoamino oxidase-B (MAO-B) enzyme breaks down DA into a stable nontoxic metabolite and ROS that protects dopamine from

intraneuronal degradation. Consequently, it decreases the metabolism of dopamine and has been found to increase dopamine levels in the brain [34].

Our data suggest that pro-inflammatory cytokines as TNF-α, IL-1β and IL-6 are incriminated with Parkinson's disease where rotenone treated rats showed significantly higher levels of such cytokines. Present results were coincide with Mogi et al. [35] and Abdel-Salam et al. [36]. It has been well established that IL6, TNF-α and IL-1β stimulate each other's secretion and exhibit overlapping and synergistic effects [37-39]. For instance, TNF-α enhances migration of leucocytes in inflamed tissue and promotes apoptosis, IL-1β acts as a potent pyrogen and decreases the threshold of pain by inducing the transcription of cyclooxygenase 2 enzyme, thereby enhancing production of prostaglandins E2, which is responsible for inflammatory pain and fever. This raises a possibility that inflammasomes, which catalyze IL-1β precursors, may stimulate TNF-α secretion through an indirect pathway [40]. The usage of URE in the treatment of Parkinson's induced rats revealed significant decrement in the levels of TNF-α, IL-1β and IL-6 than control +ve group. There is evidence suggesting the inhibitory function of rhynchophylline-type alkaloids of U. rhynchophylla in microglial activation in vitro [41]. Moreover, Rhynchophylline and isorhynchophylline have been shown to suppress the release of NO and proinflammatory cytokines [42]. Current data confirmed that URE has an anti-inflammatory effect on brain in vivo.

Rotenone induced Parkinsonism in hereby study, demonstrated significant ($P<0.05$) increase in NO and TOS levels while TAC and GSH were observed decreased ($P<0.05$) in comparison to control (-ve) group by (77% and 75 %). These results were parallel to those obtained by Tamilselvam et al. [43] in vitro and Zaitone et al. [23] in vivo. Current results could be explained by the high lipophilic affinity of rotenone that enables it to cross blood brain barrier. Once rotenone crosses blood brain barrier it acts on mitochondrial complex I in dopaminergic neurons and all over the brain [5]. Mitochondrial complex 1 is incriminated in phosphorylation of adenosine diphosphate to adenosine triphosphate and generation of H2O as end product of mitochondrial respiratory chain. Thus the depletion of such complex is usually incriminated or associated with oxidative stress [44]. Oxidative stress in dopaminergic neurons is closely associated with PD that associated with elevation of free radicals as NO and depletion of total antioxidant reserve and GSH [45]. Moreover, the ROS produced from complex 1 inhibition produced further inhibition of complex 1 and deprecate dopaminergic neurons from energy thus causing their damage [46]. The ROS-induced molecules that are released from damaged dopaminergic neurons can evoke microglial activation and pro-inflammation [47] thus pro-inflammatory cytokines increased as observed in our study. The treatment of Parkinson's induced rats with URE 500 mg/kg, markedly reduced NO and TOS levels whereas TAC and GSH levels were increased ($P<0.05$) than Parkinson's non treated rats. These results could be attributed to the flavanols contents and the alkaloids contents of URE that are capable for scavenging the free radicals and ROS [19] thus keeping the TAC and GSH in brain homogenate and subsequently reduced pro-inflammation in glial cells.

5. Conclusion

The usage of URE as therapeutic intervention for PD is beneficial. It achieves its effect through its anti-oxidant effect and free radicals scavenging that modulate pro-inflammatory cytokines production that constitutes main corner stones in the pathogenesis of PD.

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