Lauric acid: Its role in behavioral modulation, neuro-inflammatory and oxidative stress markers in haloperidol induced Parkinson’s disease

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Abstract: The study was designed to investigate the neuro-protection of lauric acid (LA) on haloperidol (HPD) induced Parkinson’s disease (PKD) rat model. Rats were divided into group A (normal), group B (diseased, by HPD 1mg/kg i.p. for 14 days), group C (standard treatment, levodopa 30 mg/kg), group D (vehicle coconut oil 1ml/kg), group E (LA 0.66mg/kg) and group F (LA 1.32mg/kg) for 35 days after induction of PKD. The study displayed a state of oxidative stress in the striatum of rat model of PKD as shown from the increased MDA, NO levels and the decreased superoxide dismutase levels. HPD caused an increase in tumor necrosis factor-α level, NF-κB, IL-8 mRNA expression and suppress IL-4 expression. Neuro-protection with LA attenuated the oxidative stress and changes in pro-inflammatory cytokines induced due to PKD induction. The LA treatment also showed improvement in the histo-pathology of the rats’ brain. LA also improved behavioral performances, food intake, weight gain as compared to animal of diseased group and prevented decline in motor activities (assessed Rotarod, and Beam walking test). LA showed significant neuro-protection against oxidative stress, inflammatory cytokines and behavioral changes in HPD induced rat model of PKD.

Keywords: Parkinson’s disease, dopamine, substantia nigra, neuro-inflammation, reactive oxygen species.

INTRODUCTION

PKD is a slowly progressing neuro-degenerative disease, influencing 1%, populace of 65 years, expanding up to 3%, in populace more than 80 years old (Hirtz et al., 2007). PKD is characterized by akinesia, festinating gait, resting tremor, rigidity, postural abnormalities, stooped posture and bradykinesia (Jomova et al., 2010). Clinical indications appear to be just if dopaminergic neural death surpasses a basic limit of 70–80%. In addition, motor and neuro-psychological functions became debilitated due to advancement of disease (Bartus and Johnson Jr, 2017).

The standard neuroleptic drug, haloperidol (HPD), for an extended time, used to treat distinctive psychotic diseases. Various patients may develop harmful, incapacitating side effects, including symptoms of PKD like muscle stiffness, depression, bradykinesia and tardive dyskinesia’s (Shin and Chung, 2012). HPD showed its effects by blocking the post-synaptic dopamine D₂ receptors in the mesolimbic system caused an increase of dopamine turnover by blockage of the D₂ receptors (Zaidi et al., 2016).

Levodopa (LVD) remains the gold standard to treat motor symptoms of PKD. Compared with other presented treatments, LVD is associated with the greatest improvement in motor function (Holloway et al., 2004).

Long-term treatment with LVD cause several types of motor fluctuations like dyskinesia, on and off effects, a problem categorized by unpredictable involuntary movements (Group, 2000).

The medium chain fatty acid of coconut oil is LA, having 12 carbon back-bone. It is found normally in various trees and animal fats, a noteworthy part of coconut and palm nut oil, which is 45-53%. Metabolic and physiological properties of LA demonstrate a few properties of coconut oil (McCarty and DiNicolantonio, 2016). Coconut oil is rapidly metabolized, readily ingested and LA is well transported and helps scales back the fat collection. LA shows significant antimicrobial action against gram-positive microorganisms and load of parasites and infections as confirmed by various investigations (Mumme and Stonehouse, 2015).

The aims of this study were to:
- Investigate the curative role of LA in HPD induced PKD.
- To observe changes in behavior (Sensory motor functions), inflammation and oxidative stress.
- Pharmacological effects of LA on oxidative stress markers (MDA, SOD and NO), mRNA expression of pro- and anti-inflammatory cytokines and behavioral changes in PKD.

MATERIALS AND METHODS

Animals
Total 42 male Wister rats (age 32-40 weeks), weighing (300-325g) obtained from animal research facility at “The University of Lahore” were used for this study, housed under controlled temperature (28°C±2°C) and humidity (60-70%). All animals were kept at 12h dark/light phases. The animals were nourished with water and standard pellet diet ad labium. Study protocol, animal handling was
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Fig. 1: Study design: At the start of research, 42 rats were randomly divided into 06 groups.

accompanied with the international procedures of ethical care and animals use in research, all experiments and protocols approved by the “The University of Lahore”. “Institutional Research Ethics Committee (IREC)”

Chemicals and Reagents
LA has molecular formula C₁₂H₂₄O₂, molecular weight 200.322, HPD has molecular formula C₂₁H₂₃ClFNO₂ with molecular weight 375.868 and Levodopa molecular formula 3,4-dihydroxy-L-phenylalanine and molecular weight is 197.19. All chemicals were procured from Sigma (St. Louis, Mo, USA). All reagents and chemicals were also of the utmost purity.

Experimental Design
Group A (Normal, n= 7),
Group B (Diseased, n=7). HPD 1 mg/kg was given intraperitoneally for 14 days.
Group C (Standard treatment n= 7) Levodopa, 30 mg/kg of body weight was given orally.
Group D (Vehicle treated n= 7) Coconut oil, 1 ml/kg of body weight was given orally.
Group E (Treated n= 7) LA-0.66 mg/kg was given orally for 35 days.
Group F (Treated n= 7) LA-1.32 mg/kg was given orally for 35 days.
LVD and LA were administered orally at a fixed time point daily for 5 weeks after induction of PkD. Due to mortality, the number decreased to six (n=6) rats per group till the completion of study.

Monitoring food intakes and body weights
Pre-weighed food was provided to each animal. The percentage change in weight, food intake throughout the five weeks were noted and monitored. Percentage change in body weight was calculated as:
(body weight after 5 weeks treatment/body weight before treatment) × 100 as reported earlier (Cheema et al., 2018).

Behavioral Analysis
At the beginning, nominated as phase-I, behavioral modeling of animals was done. During the phase-II and phase-III, behavioral testing was conducted and information was recorded. The animals were screened for motor disabilities utilizing the rotarod and beam walking test.

Rotarod test
The rotarod test was utilized to assess motor coordination, balance in rats and mice. Rats were placed in the test room for at least 1 hr prior to testing, it reduced the impacts of stress on animals. Animals from a similar cage were placed in separate lanes on the Rotarod rotating at 5rpm, such that animals may scroll forward to keep the balance. After the 60s on the rod, rats were put back to home cage and apparatus was scrubbed with 70% ethanol, permitted to dry between the trials. The methodology was performed in triplicate separated by 10 mins interims. The activity was monitored as mentioned before (Lundblad et al., 2003).

Beam walking test
The beam walking test is utilized for the evaluation of loco-motivity and grip strength. The rats were brought into the activity room 30 mins before beginning the test. The testing equipment was a 2.5×122 cm wooden beam raised 75.5 cm higher than the floor with wooden help. A 20×25×24 cm wooden container with a 9.5 cm located at the finishing end side of the beam. A button enacted light cradle (75 watts) was placed behind the start of beam, aided as avoidance motivations. The rats were delicately set at the edge of the Beam, facing the box and permitted to stroll to the end of the beam, the technique was repeated 3 times (Avila-Luna et al., 2018).

Samples preparation
Following behavioral testing, animals were relinquished by cervical dislocation after taking the blood samples.
through heart puncture. The blood was utilized to measure oxidative stress markers MDA, NO, SOD, inflammatory parameters like Tnf-α, Nf-xB, IL-8 and IL-4.

**Determination of lipid peroxidation**
MDA determined the measure thio-barbituric acid reactive species (TBARS). One molecule of MDA responds with two molecules of thio-barbituric acid within the acidic medium at 95°C temperature for 20 mins to make TBARS. The resultant pink item absorbance was estimated at 532 nm. Lipid per oxidation, was quantifiable as indicated by the technique of (Zeb et al., 2016).

**Determination of superoxide dismutase (SOD) activity**
The quantity measure depends on intensity of the enzyme to hamper the scavenging effect on superoxide anion radicals was assessed using the NBT reduction method with some modifications. Absorbance of the resulting mixture was read at 560 nm against a blank. The activity was monitored as mentioned before (Tang et al., 2007).

**Determination of nitric oxide (NO)**
Nitric oxide was determined calorimetrically. It was frequently founded, that estimating of endogenous group conc. a NO generation marker. It relies upon the adding of Griess reagent, that changes group into a purple radical aggravate whose absorbance was perused at 540 nm (Khadrawy et al., 2017).

**Evaluation of mRNA expression levels of TNF-α, NFkB, IL-4 and IL-8**
Blood was collected on day 56 of the investigation, RNA abstraction was done through the TRIzol system, getting abstraction was done through the TRIzol system, getting absorbance was perused at 540 nm. The slides were analyzed by pathologist in a blind manner.

**Assessment of Hematological and biochemical markers**
At day 56, the hematological samples were collected via heart puncture, inflammatory cells, for example, WBC count, neutrophils, eosinophil’s, lymphocytes, platelets were evaluated via hematology analyzer, creatinine and urea levels were likewise investigated by utilizing chemistry analyzer (Humalyzer 3500).

**Brain histopathology**
The rats were decapitated, brain tissue containing the substantia nigra was separated. Mounted tissues were got dried out through ascending grades of ethanol to absolute ethanol. They were purged in xylene, impregnated and installed in paraffin wax (softening point 56°C). The segments were de-waxed in xylene, hydrated through dropping descending grades of ethanol. Staining of 5-um thickness with hematoxylin and eosin staining (H&E). The slides were analyzed by pathologist in a blind manner.

**STATISTICAL ANALYSIS**
The outcome of the study was analyzed statistically by employing analysis of variances (ANOVA) Two way using Newman-Keuls multiple comparisons test, and analysis of variances (ANOVA) using Tuky test with level of significance, 0.05 using Graph pad prism ver. 7.0.

**RESULTS**

**LA improves food intake and Body weights**
Fig. 2. It shows results of cumulative 2(a) body weight 2(b) cumulative food intake of groups A (normal), group B (diseased), group C (negative control), group D (Positive control), group E (LA-0.66 mg/kg), group F (LA-1.32 mg/kg). Treatment effect measured by comparing the means of each group with other groups. Data were expressed as mean ± S.E.M. for 6 rats in each group. ANOVA, F (5, 36) = 88.38, P<0.0001 and F (5, 25) = 31.78, P<0.0001, represented by the P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 respectively. Newman-Keuls multiple comparisons posttest week 1-week 5, confirmed the significant increase in food intake and also the body weights among the treated groups and also between the time points.

**LA improves Falling Latency and Speed at fall (Rota rod Test)**
Fig. 3. show the effects of treatment with LA (0.66 and 1.32 mg/kg) on speed at fall 3(a) and latency to fall 3(b), for 5 weeks. Values are means ± SD (n = 6). Substantial differences by Newman-Keuls multiple comparisons: *P < 0.05 following two-way ANOVA (repeated measure design).

It shows results of Rota rod test 3(A) speed at fall and 3(B) falling latency, A (normal), group B (diseased), group C (negative control), group D (Positive control), group E (LA-0.66mg/kg), group F (LA-1.32mg/kg). Treatment effect measured by comparing the means of each group with other groups. Data were expressed as mean ± S.E.M. for 6 rats in each group. ANOVA, F (5, 757)
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LA improves Time and slip latency (Beam walking test)
Fig. 4. Shows the effects of treatment with LA treatment dosage (0.66mg/kg and 1.32mg/kg) on group E and F on different parameters of beam walking test speed 4(A) and slip latency 4(B). Values are means ± SD (n=6). Significant differences by Newman-Keuls multiple comparisons.

Fig. 4: Beam walking test (A) Time and (B) slip latency

LA Normalized Biochemical and hematological Markers
Fig. 5 LA stabilized biochemical parameters such as 5 (A) Creatinine, 5 (B) Urea, 5 (C) and like hematological stricture, WBC, 5 (D) Lymphocytes and 5 (E) Neutrophils count 5 (F) Platelets count also considerably attenuated with treatment (0.66mg/kg and 1.32mg/kg) in group E and F (n=6), as compared with group C, D and disease group B.
LA improves the MDA, SOD and NO levels

Fig. 6 Effects of repeated administration of LA (0.66mg/kg and 1.32 mg/kg/day) for 5 weeks reduces lipid peroxidation and showed improvement in (A) SOD (B) MDA and (C) NO levels in groups E and F as compared to disease group (group B). Values are means ± SD (n = 6). Significant differences by Tukey’s test: shows noteworthy (****) change compared to group B (####) indicates significance compared to standard treatment, vehicle and treatments group following two-way ANOVA (repeated measure design).

LA suppress mRNA expression levels of TNF-α, NFκB and IL-8 and improve IL-4 expression

Fig. 7 Graphical representation of mean ± SD relative expression levels of 7(A) TNF-α, 7(B) IL-8 and 7(C) NFκB suppressed in LA (0.66 and 1.32mg/kg) E and F groups. However, LA treatment causing improvement in
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Fig. 7: mRNA expression levels of TNF-α, IL-8, NFκB and IL-4 expression

Significantly raised (P<0.0001) levels of TNF-α found in group B as compared to standard treatment, vehicle, and treated groups E (P=0.0387) and F (P=0.9961) Treatment with LA-0.66mg/kg (0.5337± 0.21797; P<0.05) and LA-1.32mg/kg (0.5881±0.2401; P<0.01) considerably suppressed mRNA expression levels of TNF-α (fig. 7). It was also found that, significantly raised (P<0.01) levels of NFκB expression in treatment groups E (1.8239±0.2466) and F (1.0991±0.2466) as compared to group B (3.4883±0.4961). Similarly, IL-8 mRNA expression also suppressed in treatment groups E (2.3209±0.1502) and F (2.776±0.3030) shows significantly up regulated IL-4 levels (P < 0.05) (fig. 8).

Histo-pathological Evaluation

Figure 8 Photomicrograph of A- (normal group) representing normal axons and neurons. B (disease group) showing loss of noticeable axons and Lewy bodies in numbers, C (treated with levodopa) showed disturbed neurons but intact neurons were also present. D (vehicle treated) with coconut oil showed normal neuronal cells but also the cells with Lewy body. E (treated with 0.66mg of LA) showed intact parenchyma and few neurons. F (treated with 1.32 mg/kg of LA) showed normal neurons and stable parenchyma (100×, H&E stain). Histo-pathological evaluation (fig. 8) showed, the damaging effect of HPD on the rat brain as atrophic neurons, disturbed parenchyma and Lewy bodies. The treatment groups E, F showed, an increased in body weight. In addition, the rats treated with levodopa group C or vehicle (coconut oil) group D also improve the motor functions of the animals but are less significant in comparison with LA treatment groups. The other objective of this study to evaluate the effects LA on HPD-induced changes towards the anti-oxidant levels in rats. The dosing of LA to HPD induced PkD rats, nearly restored the activity of SOD in serum, therefore confirmed a distinctive defensive effect in histo-pathological evaluations. The significant raise in anti-oxidant activity joined with the histo-logical confirmation, leads to assumption that, LA reduces HPD-induced oxidative impairment in rat brain. The black arrows point to normal neurons in group A rat substantia nigra section, showing normal neuronal pathology of the SN of control rats with no histo-pathological changes having normal dopaminergic neuronal population. The magnified image indicates the damaged and presence of “Lewy bodies” in “substantia nigra” sections of group B, C and D. However, group E and F treated with different doses of lauric acid showed much improvement in concentration of Lewy bodies and damage in substantia nigra sections compared to the sections of “substantia nigra” of group B, C and D.

Docking with D₂ Receptor

Fig. 9. During this study, dopamine D₂ receptor docked with 9(A) Haloperidol, 9(B) Levodopa and 9(C) Lauric
acid using Autodock soft-ware. The docking method, Molecular docking studies has shown that Lauric acid having potential to dock with dopamine D2 receptor showing good binding energy levels in comparison with HPD and LVD.

and target protein was evaluated on binding energy score, RMSD values by auto Dock.

**Fig. 9:** Molecular docking of HPD (A), LVD (B) and LA (C) with D2 receptor

Outline of the molecular docking process. Three-dimensional structure of the ligand (Haloperidol, Levodopa and Lauric acid); Three-dimensional structure of the receptor Dopamine D2. The ligand (haloperidol, levodopa and Lauric acid) binds with specific site on specific chain of D2 receptor; The ligand is docked into the binding cavity of the receptor and the putative conformations are explored; The most likely binding conformation and the corresponding intermolecular interactions are identified; shows the binding energy of ligand (haloperidol, levodopa and lauric acid) on D2 receptor. The protein backbone is represented as a cartoon. The ligand is shown in stick representation and hydrogen bonds are indicated as dashed lines.

**Fig. 10:** Binding energies of HPD, LVD and LA

**DISCUSSION**

This research study was aimed to screen the long-term administration of LA effects, minimizing the parkinsonian effects developed through chronic administration of HPD blocking the brain DA, affecting the behavior. A consistent finding of this study was, increase in weight, food intake as well as muscular strength of group E and F group rats. Both the dosage of LA improved performance in beam walking and rotarod test in treated animals as compared to animal of group B, C and D. Moreover, animals treated with Vehicle also showed some improvement in food intake and weight gain.

In rotarod test, rats’ groups E and F treated with different doses of LA, tested for speed at fall, falling latency in a specific environment showed improvement compared with groups B, C, D treated with HPD, LVD and Vehicle. This week-to-week improvement in behavior of groups E and F was dose dependent showed significance (P<0.0001), compared to group B.

The motor-function deficiencies was detected on beam walking test in groups B, compared with groups C, D treated with LVD and Vehicle however significant improvement was shown by rats of groups E and F treated with different dosing of LA in time latency to complete the task and number of slips while completing the task. Dosing regimens of HPD were conveyed to exhibit deficiencies, in stride-length, motor and limb coordination, after two weeks post-HPD administration. In addition, HPD-induced rats increase in time extent to cross beam compared to rats treated with different dosage of LA. Data analysis showed that animals of group B display hind-limb weakness, freezing behavior, (akinesia) late motor initiative as compared to treated C, D, E and F group rats. The group B showed increased slip and time latency to cross the beam as well as number of slips in comparison with rats treated with different dosage of LA (P<0.0001).
In this study, we demonstrate that i.p injection of HPD for 14 days induced PkD symptoms and increase in MDA, NO levels. This was related with a substantial reduction in SOD activity. Present findings indicate the development of a state of oxidative-stress caused by HPD. The creation of ROS which, in turn, caused by lipid-peroxidation, mitochondrial and DNA damage (Angelova and Abramov, 2018). Worsening this situation were the vulnerability of brain to oxidative-stress due to high oxygen consumption and high content of poly-unsaturated fatty acids that are predominantly exposed to free radical attack (Kim et al., 2015). Thus, the significant increase in level of MDA in group B rats attributed to attack of the cell of the brain by the free radicals evolved by lipid per oxidation. However, there was marked reduction in MDA showed by group E and F (P<0.0001), (LA treated groups) compared to group B.

This study clearly demonstrates neuro-protectiveness of LA, ameliorated escalation in lipid peroxidation induced by HPD. The ability of LA to restore SOD activity, may explain the recovery of damage initiated by lipid peroxidation. The recovery of NO (P<0.0001), after LA treatment may arise from the inhibition of NOS as compared to results early described in research, since NO derived from activated glial cells were assumed to contribute to neuronal death during neurodegenerative diseases like PkD (Phatnani and Maniatis, 2015). The result of this study suggested that LA able to prevent lipid peroxidation. Supporting this antioxidant activity of LA was due to increase in SOD activity in rat treated with LA. This was in agreement with the study of which observed that caffeine promotes SOD activity (Khadravy et al., 2017). Thus, the significant increase in the present SOD activity could be attributed to antioxidant activity of LA (P<0.0001), compared to group B. It may be endorsed to the scavenging of superoxide anion radicals and the enhancement of enzyme activity by LA. This effect could be explained on the basis that LA decreased the progression of disease by inhibiting free radical formation and the inflammatory process caused by HPD.

Neuro-inflammation represents one of the successive events underlying the development of dopa-minergic neuro degeneration (Tansey and Goldberg, 2010). It has been reported that the activated microglia-induced increase in levels of TNF-α plays a part in dopaminergic neuro-degeneration (Montgomery and Bowers, 2012). Consequently, the present increase in TNF-α, NFkB, IL-8 is a potent pro-inflammatory cytokine as shown by earlier studies (Larsson., et al 2015) and decrease in IL-4 an anti-inflammatory cytokine, in the blood of rat model of PkD is an indicator of the neuro inflammation induced by HPD. However, the treatment with LA in E and F groups shows significant improvement in mRNA expression of TNF-α, NFkB, IL-8 as compared to standard treatment vehicle and disease groups. LA also improved the mRNA expression of IL-4 in comparison with other treated groups in the study.

The histo-pathological investigation in HPD induced rats showed neuronal degeneration in the two areas that were characterized by the existence of cytoplasmic inclusions of Lewy bodies, gliosis in striatum of the brain. These histo-pathological changes may result from the chronic HPD-induced blockade of D3 receptors which in turn cause mitochondrial dysfunction, energy crisis, oxidative stress, and neuro inflammation mediated by activated microglia. The decrease in the striatal dopaminergic activity may underlie the observed changes in the locomotor activity of PkD rats. These changes in motor activity together with neuro-chemical, histo-pathological changes indicate the establishment of the rat model of PkD.

**CONCLUSION**

In conclusion, the present study supports neuro-protective use of LA in improving motor symptoms and general behavior of rats with Parkinson’s disease. The study focuses on underlying mechanisms that suppression of ROS and pro-inflammatory cytokines levels improved sensori-motor function may and may halt the progression of PkD in LA treated groups. The neuro-chemical, histo-pathological, behavioral findings and molecular docking of our study demonstrated the neuro-protective effectiveness of LA. Moreover, this study shows that particularly lower dose of LA improves motor activity, produce beneficial effects on behavioral functions, immune modulation and suppression of ROS, and provide protection against dopaminergic neurons degeneration.

**REFERENCES**


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