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Comparative influence of Kolaviron and Coenzyme Q10 on Complex I activity, Glutamate clearance, 3,4-Dihydroxyphenethylamine metabolism and redox stress in Rotenone-induced Neurotoxicity

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Summary: 3,4-dihydroxyphenethylamine (dopamine) depletion, inhibition of complex I activity, oxidative stress, and glutamate excitotoxicity are cardinal biochemical features of neurotoxicity induced by systemic unilateral infusion of rotenone. Kolaviron (KV), a biflavonoid from *Garcinia kola* seeds, has been proven to have pharmacological effects against neurotoxicity. Coenzyme Q_{10} plays an essential role in mitochondrial oxidative phosphorylation and as an antioxidant. This study examined the comparative influence of kolaviron and coenzyme Q_{10} on complex I activity, dopamine metabolism, glutamate clearance, and redox stress in rotenone-induced neurotoxicity in the cortex, hippocampus, and striatum of the brain of rats. Adult Male Wistar rats were pretreated with 200 mg/kg KV or 100 mg/kg coenzyme Q_{10} for 7 days followed by administration of a progressive six doses of 1.5 mg/kg rotenone within the next 48 h after which the animals were euthanized and the brain excised. On the cortical, hippocampal, and striatal regions of the brain, complex I activity, dopamine metabolism, oxidative stress markers, as well as glutamate metabolism were carried out and analyzed. In all brain regions examined, KV and coenzyme Q_{10} pre-treatment modulated complex I activity, ameliorated redox imbalance, and enhanced dopamine metabolism via increasing the activity of tyrosine hydroxylase and decreasing monoamine oxidase activity. KV facilitated glutamate clearance through augmentation of glutamate dehydrogenase and glutamine synthetase activities. The activity of KV was comparable to that of the mitochondrial membrane antioxidant compound, coenzyme Q_{10}, this indicates that KV is a promising therapeutic agent in the treatment of Parkinson's disease and its activity compares well with coenzyme Q_{10}.

Keywords*: Rotenone; kolaviron; coenzyme Q_{10}; 3,4-dihydroxyphenethylamine; complex I; glutamate*

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INTRODUCTION

Pesticides are substances that are being used to control unwanted pests and overexposure to these pesticides can lead to neurotoxicity, which can cause progressive loss of structure and/or functions of neurons such as in Parkinson's disease. Parkinson's disease (PD) is a neurodegenerative disorder characterized by selective degeneration of dopaminergic neurons in the substantia nigra leading to a reduction of dopamine levels in the brain (Paul *et al*., 1992). PD is the second leading cause of death in age-related neurodegenerative diseases (Ira *et al*., 2006). 3,4-dihydroxyphenethylamine, also known as dopamine, is an important neurotransmitter mediating a variety of higher brain functions such as movement, memory, pleasurable reward, behavior, and cognition. Brain dopamine levels must be carefully regulated to avoid neuronal instability or over-activation (Suri and Schultz, 1999; Suri *et al*., 2011; Hyman *et al*., 2006). In addition, altered cerebral glutamate metabolism is a key feature of PD and abnormal patterns of glutamatergic neurotransmission are important in the symptoms of the disease (Bladini *et al*., 1996). Increased metabolism of neuronal glutamate in dopamine-depleted striatum has been reported (Chassain *et al*., 2005). Mitochondrial dysfunction has also been linked to PD, specifically through systemic reductions in the activity of complex 1 of the mitochondrial electron transport chain. Inhibition of complex 1, the incidence of glutamate excitotoxicity, and most especially, oxidative damage as a result of oxidative stress through the generation of reactive oxygen species and free radical formation contribute to the neurodegenerative process in PD (Jenner, 1998; Onyema *et al*., 1998; Sherer et., 2001).

Epidemiological studies have suggested that exposure to environmental agents such as pesticides such as rotenone, may increase the risk of PD (Gorell *et al*., 1998). Symptoms of Parkinsonism include tremors at rest (Bladini *et al*. 1996), rigidity (increased stiffness) and bradykinesia (slowness or absence of voluntary movement) (Greenamyre and Hastings, 2004), postural instability, cognitive changes (memory problems, personality changes, hallucinations) and loss of appetite (Chou *et al*., 2013; David *et al*., 2012). Rotenone is a commonly used pesticide and is a potent specific inhibitor of mitochondrial complex I. Systemic unilateral infusion of rotenone produces neurochemical and neuropathological features of Hemi-Parkinsonism in vertebrates with the involvement of oxidative stress as a major mechanism (Betarbet *et al*., 2000). It is therefore used to mimic the symptoms and characteristics of Parkinsonism in studies to unravel the mechanisms behind neurodegeneration in PD (Betarbet *et al*., 2000; Thiffault *et al*., 2000).

Therapeutic agents for the treatment and management of PD are very limited. Kolaviron (KV), a well-characterized biflavonoid from *Garcinia kola* seeds consists of biflavanones (GB1, GB2, and kolaflavanone) and has been reported to demonstrate antioxidant, anti-inflammatory, neuroprotective, and neurostimulatory properties (Adegboye *et al*., 2008; Farombi and Owoeye, 2011; Farombi *et al*., 2013; Akinmoladun *et al*., 2015). Its effectiveness centers on its ability to act on multiple targets in pathological processes. Coenzyme Q_{10} (Ubiquinone), an electron acceptor for complexes I and II, has been reported to possess neuroprotective effects in multiple *in vitro* and animal models of neuronal toxicity (Jie, 2014). KV has been reported to be useful in the management of Parkinson's disease and its related behavioral abnormalities (Farombi *et al*., 2019; Farombi *et al*., 2020), but there is no study that defines the comparative evaluation and advantage of KV and coenzyme Q_{10} in rotenone-induced PD. The aim of this research was to assess the comparative pharmacological potential of kolaviron and coenzyme Q_{10} in rotenone-induced neurotoxicity in rats' discrete brain regions.

MATERIALS AND METHODS

Chemicals and Reagents: Rotenone, , adenine triphosphate (ATP), nicotinamide adenine dihydronuclotide (NADH), coenzyme Q_{10}, 6,7-dimethyl-5,6,7,8-tetrahydropterine (DMTHP), 5',5'-dithiobis-(2-nitrobenzoic acid) (DTNB), benzylamine hydrochloride (BAHC), sodium dodecyl sulphate (SDS), ethylenediaminetetraacetic acid (EDTA), 1-amino-2-naphtol-4-sulphonic acid (ANSA), 2,4-dinitrophenyl hydrazine tetramethylbenzidine (TMB) and ammonium molybdate were obtained from Sigma-Aldrich (St-Louis, MO, USA). All other chemicals and reagents used were of analytical grade. The assay kit used for total protein determination was obtained from Teco laboratories, Philadelphia, USA.

Extraction of Kolaviron: Extraction of KV was achieved by the procedure previously described by Iwu (Iwu 1985), and modified by Farombi *et al*. (2000). Briefly, *Garcinia kola* seeds were peeled, pulverized with an electric machine, and dried in the laboratory at room temperature. The powdered seeds were extracted with light petroleum ether (b.p. 40-60 ºC) in a Soxhlet extractor for 24 h. The defatted; dried marc was repacked and then extracted with acetone. The extract was concentrated and diluted to twice its volume with distilled water and partitioned with ethyl acetate. The concentrated ethylacetate fraction yielded a yellow-brown residue which was kolaviron.

Experimental Design: Male Wistar rats weighing 200 to 230 g that were bred and housed at the Animal House of the Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria were used for the experiment. Animals were fed with laboratory chow (Vital Feeds, Lagos, Nigeria) and water *ad libitum* and were handled and used in accordance with the international guidelines for the care and use of laboratory animals (NIH, 1985). Animals were divided into 5 groups (n=8) as shown below. KV and coenzyme Q_{10} were dissolved in corn oil (vehicle). KV was orally administered to animals (p.o.) for 7 consecutive days before systemic subcutaneous (s.c.) administration of 1.5 mg/kg rotenone 6 times within 48 h according to the method of Thiffault *et al*. (2000). Dosage of KV (200 mg/kg) and coenzyme Q_{10} (100 mg/kg) used were based on previous studies (Abd-El Gawad e al., 2004; Akinmoladun *et al*., 2015; Farombi *et al*., 2019).

Group I received 1 ml/kg corn oil (Control); group II received rotenone only (Rotenone); group III received 200 mg/kg KV and rotenone (Rot + KV (200 mg/kg)); group IV was administered 100 mg/kg coenzyme Q_{10} and rotenone (Rot + Ubiquinone); and group V was administered 200 mg/kg of KV only (KV only (200 mg/kg)).

At the end of the period of treatment, animals were euthanized, the brain was excised and hippocampi, striata, and cortices were separated and processed for biochemical evaluations.

Biochemical Estimations: Hippocampi, striata, and cortices from excised brains washed in ice-cold 1.15% (v/v) potassium chloride solution blotted with filter paper, weighed and separately homogenized in 10% (w/v) phosphate-buffered saline PBS (pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 *g* at 4 ºC for 25 min to obtain the supernatant (test sample) used for biochemical analyses. Total protein was determined using the Randox assay kit and the manufacturer's instruction was followed. The method was based on Weichselbaumin (1946).

Evaluation of Complex 1 activity: The activity of complex I was evaluated according to the method of Birch-Machine *et al*. (1994). The results were expressed as changes in optical density/mg protein

Evaluation of glutamate clearance markers: The method of Sadasivam and Manickam (2003) and Akinmoladun *et al*. (2015) were followed for evaluating the activity of glutamine synthetase (GS). The activity of glutamate dehydrogenase (GDH) was carried out according to the method described by Abdel-Zaher *et al*. (2011).

Evaluation of dopamine metabolism markers: Tyrosine hydroxylase activity was measured by the method described by Shiman *et al*. (1971) and Crane *et al*. (1972). The concentration of dopamine was evaluated as described by

Guo *et al*. (2009). Monoamine oxidase activity was measured by using the method developed by Holt *et al.* (1997) and described by Chaudhary and Parvez (2012).

Estimation of markers of oxidative stress: The method of Beutler (1963) was followed in estimating the level of reduced glutathione (GSH). Glutathione peroxidase activity was determined according to the method of Rotruck *et al.* (1973). Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972). The level of lipid peroxidation was evaluated by measuring the formation of TBA reactive substances (TBARS) according to the method of Ohkawa *et al*. (1979). Xanthine oxidase activity was measured using the method of Prajda and Weber (1975). Protein carbonyl content was determined according to the method of Levine *et al*. (1990).

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation and oxidative stress was evaluated according to the method of Eiserich *et al*. (1998).

Statistical analysis

Results were expressed as mean ± SD. Statistical differences between means were determined by One–Way analysis of variance followed by Duncan's posthoc multiple comparison tests. $P < 0.05$ was considered statistically significant. The statistical software used to analyze the data was GraphPad Prism 6.0 (GraphPad Software Inc., CA, USA).

RESULTS

Kolaviron and coenzyme Q_{10} modulated striatal complex 1 activity in the brain of rotenone-intoxicated rats: Fig. 1 shows a significant ($p < 0.05$) reduction in complex 1 activity in the cortex, striatum, and hippocampus of rotenone-toxified rats compared with control.

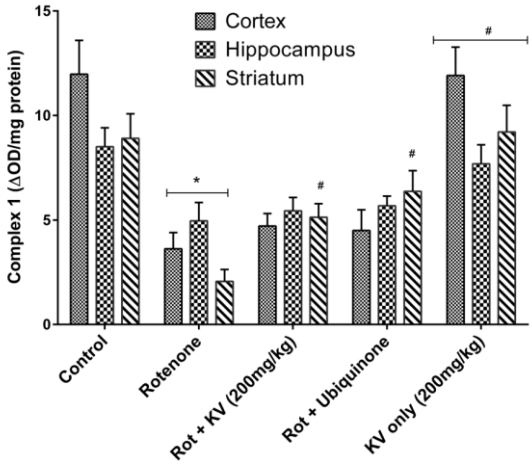

Figure 1:
Effect of kolaviron and coenzyme Q_{10} on complex 1 activity in the cortical, hippocampal, and striatal regions of the brain of rotenone-toxified rats. Each representssent mean ± SD (n=8). *$p < 0.05$ vs control; $^{\#}p < 0.05$ vs Rotenone. Rot: Rotenone; KV: Kolaviron

Rats pretreated with KV and coenzyme Q_{10} only showed a marked increase ($p < 0.05$) in striatal complex I activity compared with rotenone-toxified rats but did not have an effect on cortical and hippocampal complex I activities. Pretreatment with KV did not show any significant difference with coenzyme Q_{10} pre-treatment.

Kolaviron and coenzyme Q_{10} enhanced 3,4-dihydroxyphenethylamine metabolism in brain regions of rats exposed to rotenone: Tyrosine hydroxylase activities, 3,4-dihydroxyphenethylamine levels, and monoamine oxidase activities in the three brain regions of rats exposed to rotenone are depicted in Fig. 2.

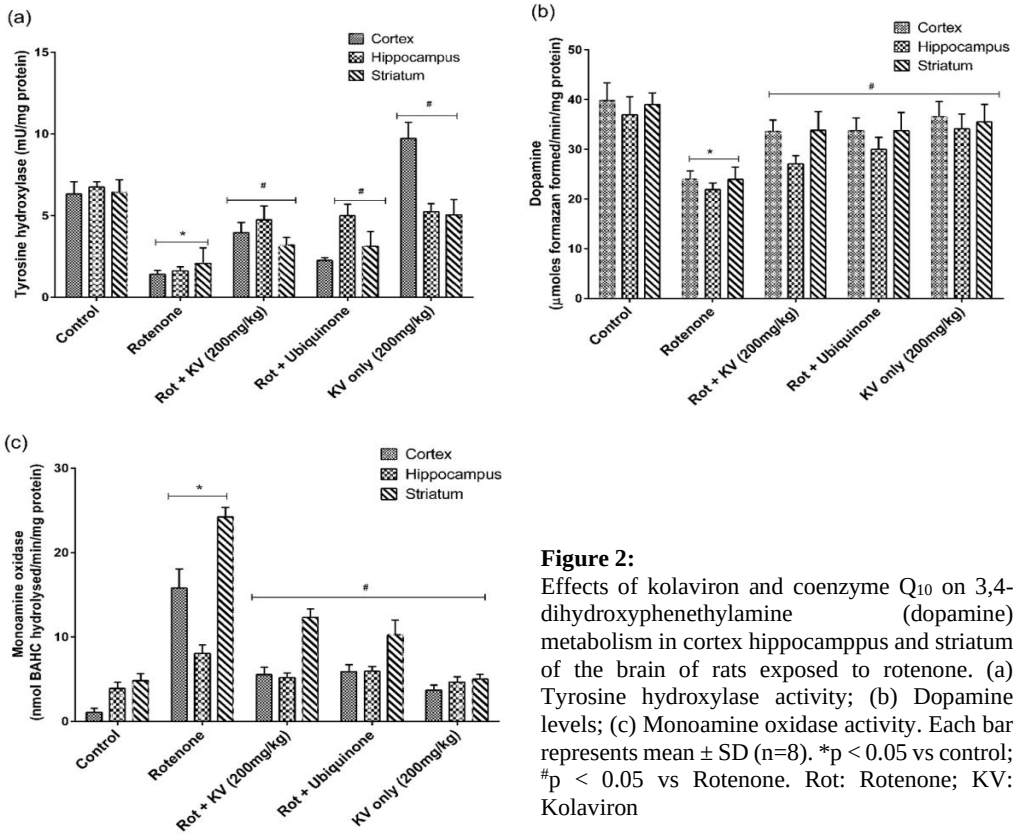

Figure 2:
Effects of kolaviron and coenzyme Q_{10} on 3,4-dihydroxyphenethylamine (dopamine) metabolism in cortex hippocamppus and striatum of the brain of rats exposed to rotenone. (a) Tyrosine hydroxylase activity; (b) Dopamine levels; (c) Monoamine oxidase activity. Each bar represents mean ± SD (n=8). *$p < 0.05$ vs control; $^{\#}p < 0.05$ vs Rotenone. Rot: Rotenone; KV: Kolaviron

Rotenone significantly ($p < 0.05$) reduced the activities of tyrosine hydroxylase (Figure 2a) and 3,4-dihydroxyphenethylamine levels (Fig. 2b) level, as well as significantly ($p < 0.05$) increase the activities of monoamine oxidase (Fig. 2c) in the cortical, striatal and hippocampal regions of rats brain. Rats pre-treated with KV and coenzyme Q_{10} significantly showed enhanced 3,4-dihydroxyphenethylamine metabolism in all the brain regions by increasing the activity of tyrosine hydroxylase and decreasing the activity of monoamine oxidase. Pre-treatment with KV only showed a marked increase in cortical tyrosine hydroxylase activity compared with pre-treatment with coenzyme Q_{10}.

Kolaviron and coenzyme Q_{10} facilitated cortical, hippocampal, and striatal glutamate clearance in rotenone-induced neurotoxicity: Fig. 3 depicts the activities of glutamine synthetase and glutamate dehydrogenase in the cortex, hippocampus, and striatum of rotenone-induced rats. There was a significant ($p < 0.05$) decrease in the activities of glutamine synthetase (Fig. 3a) and glutamate dehydrogenase (Fig. 3b) in the cortical, hippocampal and striatal regions of rotenone-induced rats compared with the control. This leads to glutamate excitotoxicity. KV and coenzyme Q_{10} pre-treatment significantly ($p < 0.05$) facilitated glutamate clearance through augmentation of the activities of glutamate dehydrogenase and glutamine synthetase in the cortex, hippocampus, and striatum of rotenone-induced rats.

Kolaviron and coenzyme Q_{10} blunted redox imbalance and MPO activity in cortex, hippocampus, and striatum of rotenone-intoxicated rats: Fig. 4 revealed that oxidative stress was elicited in the cortical, hippocampal, and striatal regions of the brain of rotenone-toxified rats compared with the control ($p < 0.05$). Reduced glutathione levels (GSH), Glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities were significantly ($p < 0.05$) decreased in the cortex, hippocampus, and striatum of rats intoxicated with rotenone (Fig. 4a-c). Pretreatment with KV and coenzyme Q_{10} significantly ($p < 0.05$) mitigated rotenone-induced redox imbalance by increasing GSH levels in all the examined brain regions, increasing the activity of SOD and cortical and striatal GPx. Rats pretreated with coenzyme Q_{10} showed no effect on hippocampal SOD and GPx compared with rotenone intoxicated rats. Striatal GPX activity increased markedly in KV pretreated rats compared with coenzyme Q_{10} pretreatment. Furthermore, lipid peroxidation (Fig. 4d) and protein carbonyl (Fig. 4e) levels were significantly elevated in the cortex, hippocampus, and striatum of rotenone-induced rats compared with the control ($p < 0.05$). KV and coenzyme Q_{10} ameliorated rotenone-induced oxidative damage significantly ($p < 0.05$) by reducing the levels of lipid peroxidation and protein carbonyl in all the brain regions except KV pretreated hippocampal protein carbonyl level which showed no change compared with the rotenone-toxified hippocampal region. Also, an increase in the activity of xanthine oxidase (Fig. 4f) in rotenone-toxified rats was markedly decreased by KV and coenzyme Q_{10} in all the regions under examination. Myeloperoxidase activity in Fig. 5 in all regions of rotenone-intoxicated rats was significantly increased ($p < 0.05$) compared with the control. This signifies that inflammatory events are initiated. The increase in myeloperoxidase activity by rotenone was significantly attenuated by KV and coenzyme Q_{10}. Coenzyme Q_{10} showed a better cortical MPO ameliorative ability than KV

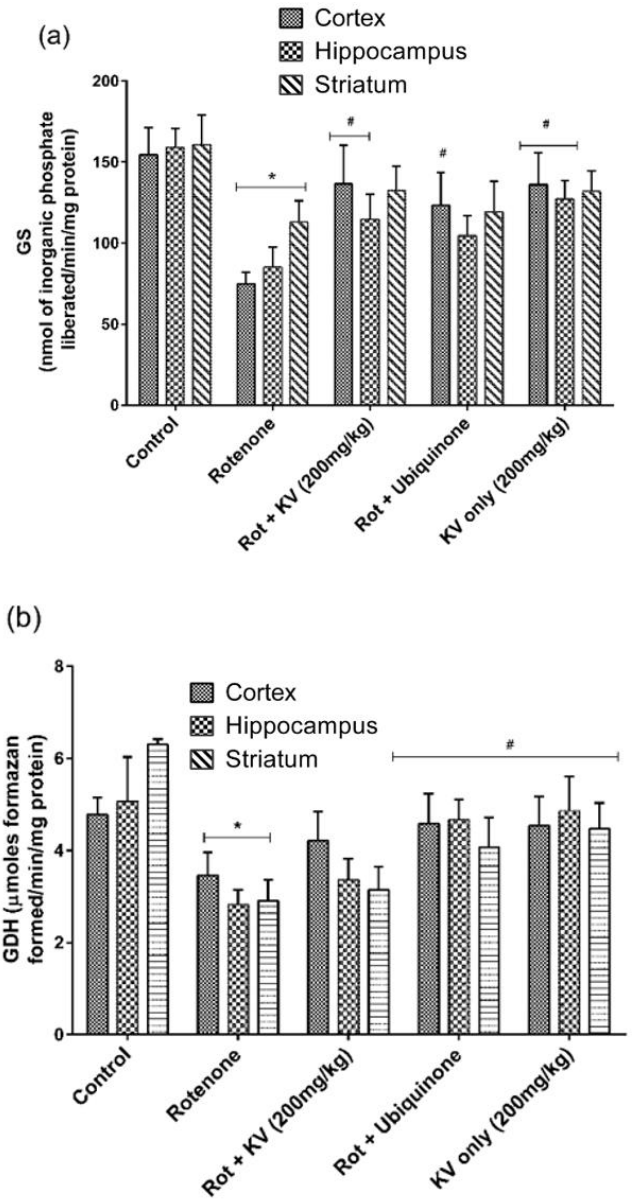

Figure 3:
Effects of kolaviron and coenzyme Q_{10} on cortical, hippocampal, and striatal glutamate clearance in rotenone-induced neurotoxicity. (a) Glutamine synthetase (GS) activity; (b) Glutamate dehydrogenase (GDH) activity. Each bar represents mean ± SD (n=8). *$p < 0.05$ vs control; #$p < 0.05$ vs Rotenone. Rot: Rotenone; KV: Kolaviron.

DISCUSSION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders globally, affecting 1% of the population older than 65 years of age. Previous studies have found that most causes of PD are due to environmental toxins and the mechanism involved is the inhibition of mitochondrial complex 1 (Muzuno *et al*., 1998). Rotenone, a natural biopesticide crosses the blood-brain barrier (BBB) and other biomembranes easily due to its lipophilic nature.

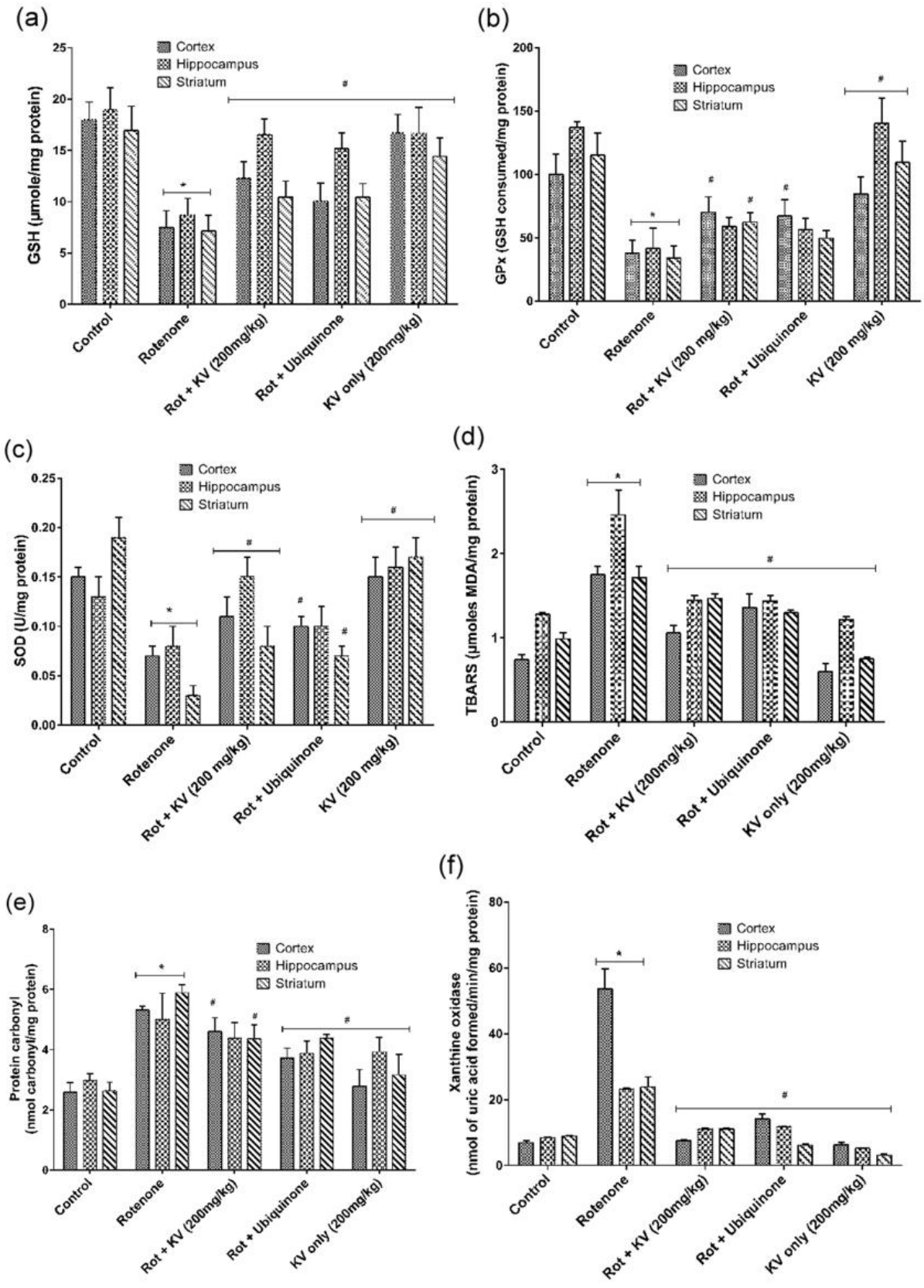

Figure 4:
Effects of kolaviron and coenzyme Q10 on redox imbalance in the cortex, hippocampus, and striatum of the brain of rotenone intoxicated rats (a) Reduced glutathione (GSH) levels; (b) Glutathione peroxidase (GPx) activity; (c) Superoxide dismutase (SOD) activity; (d) Lipid peroxidation levels (TBARS); (e) Protein carbonyl levels; (f) Xanthine oxidase activity. Each bar represents mean ± SD (n=8). *p < 0.05 vs control. *#p < 0.05 vs Rotenone; Rot: Rotenone; KV: Kolaviron*

The high lipoliphicity of rotenone enables it to easily cross biological membranes including the BBB (Higgins and Greenamyre 1996). Rotenone inhibits the activity of mitochondrial complex I in the dopaminergic neurons, causing alterations to dopamine and glutamine metabolism, as well as redox imbalance and mitochondrial dysfunction (Cannon and Greenamyre 2013; Terron *et al*., 2018). Administration of rotenone for 48 h leads to 'parkinsonism' a syndrome characterized by different neurological and neurobehavioral deficits which correlate with Parkinsonian symptoms (Prasad and Hung 2020). KV has been reported to be a promising antioxidant that could be useful in the management of Parkinson's disease (Farombi *et al*., 2019; Farombi *et al*., 2020) and clinical evidence of phase II trial of coenzyme Q_{10} (ubiquinone) against PD has shown that administration of coenzyme Q10, which is an antioxidant, slows the progressive loss of functional deficit seen in PD (Shults *et al*., 2020) but there is no research in the comparative evaluation between KV and coenzyme Q_{10} to know if one of the antioxidants could perform better than the other in preclinical studies.

In this study, administration of rotenone caused a biochemical change to dopamine metabolism in cortex, hippocampus, and striatum of the brain of rats with the most damage seen in the striatal region. This cemented the claim that systemic infusion of rotenone selectively caused nigrostriatal degeneration of dopaminergic neurons in Parkinson's disease (Betarbet *et al*. 2000; Farombi *et al*., 2019). Also, the involvement of other brain regions in the etiology of functional deficit seen in Parkinson's disease was evident through the evaluation of biochemical changes in this study. Mitochondria protecting the ability of KV and coenzyme Q_{10} was revealed in this study when it ameliorated the cortical, hippocampal and striatal reduction of complex 1 activity in the rotenone-toxified rat. KV and coenzyme Q_{10} reduced alteration in dopamine metabolism through modulation of tyrosine hydroxylase and monoamine oxidase activities and dopamine level. Along with other reports, this suggests that KV slowed dopamine alterations in the rotenone-toxified rat. Perturbation of glutamate has been reported in models of Parkinson's disease (Lee *et al*., 2005). The present study showed that the activities of GDH and GS were reduced after rotenone intoxication and can result in glutamate excitotoxicity. GDH catalyzes the oxidative deamination of glutamate to other amino acids while GS plays a vital role in the amidation of glutamate to form glutamine (Neidhardt 1996; Nelson and Cox 2004). Pre-treatment with KV and coenzyme Q_{10} showed that it significantly ameliorates the decrease in GDH and GS activities and verifies the role of KV to reduce excitotoxicity in PD. The mitochondrial and neurotransmitter modulatory ability of both KV and coenzyme Q_{10} against rotenone-induced PD in rats is obvious from this study but no distinct difference can be marked between the two antioxidants which means their activity is comparative.

Reactive oxygen species (ROS) could be formed through multiple injury mechanisms during PD (Lee *et al*., 2003; Onyema *et al*., 2004; Fato *et al*., 2009). Oxidative stress remains a cornerstone of the concepts underlying the loss of dopaminergic neurons in PD. Lipid peroxidation can lead to membrane damage. Moreover, the end products of lipid peroxidation are toxic to neurons (Bruce-Keller *et al*., 1998), including axons and oligodendrocytes, being able to induce apoptosis (Montine *et al*., 1996; McCraken *et al*., 2000). Damage to proteins, particularly enzymes, can inactivate their function. Also, DNA oxidation can cause the activation of repair enzymes that provokes a rapid depletion of intracellular energy leading to cell death. Reduced levels of GSH and activities of antioxidant enzymes, SOD and GPx, and an increase in TBARS indicate elicitation of oxidative stress in rotenone intoxicated PD. Pre-treatment with KV and coenzyme Q_{10} mitigated oxidative stress by ameliorating these antioxidant and antioxidant enzyme markers as well as decreasing the activity of xanthine oxidase and reduction of TBARS and protein carbonyl levels. KV is an important neuroprotective agent capable of protecting the brain against neuronal damage. Also, MPO functions as a key molecular component of the host defense system against diverse pathogens. Chang *et al*. (2011) reported that increased MPO activity is a distinguishing feature of rotenone-exposed glial cells and that either over-activation or deficiency of myeloperoxidase leads to pathological conditions in the brain. KV and coenzyme Q_{10} significantly decreased the activity of the myeloperoxidase in all the regions of the brain after rotenone intoxication, this confirms the anti-inflammatory potential of KV that has been reported (Farombi *et al*., 2013; Ojo *et al*., 2019). This study clearly showed that KV and coenzyme Q_{10} ameliorated oxidative damage in rotenone-intoxicated neurotoxicity in rats and comparatively, there is no significant difference in their ability to alleviate oxidative stress.

In conclusion, this study affirms the neuroprotective potential of KV to assuage neurochemical dysfunction as a result of rotenone intoxication. Also, there is no clearly defined or significant evidence in this study that could point to the comparative difference in the ameliorative role of KV and coenzyme Q_{10} against rotenone-induced neurotoxicity in discrete rat's brain regions. Therefore, it is recommended that further/more sophisticated work should be carried out in order to distinctively characterize if they (KV and coenzyme Q_{10}) are more closely comparable in their activity.

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