Original Article

Antioxidative Potential and Activity of Potassium Polyacrylate and Coenzyme Q10 on Rat Hepatic Mitochondrial Permeability Transition Pores

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ABSTRACT

Multiple biological activities of coenzyme Q10 have been demonstrated, opening up opportunities for research and development. However, the biological action of potassium polyacrylate and its effect on the mitochondrial permeability transition pores are both poorly understood. Therefore, this study investigated the *in vitro* antioxidative potential of potassium polyacrylate (PCK) and coenzyme Q10 (CoQ10) and their effects on mitochondrial permeability transition pores. *In vitro* antioxidant and angiotensin-converting enzyme inhibitory activities were assessed using standard methods, and lipid peroxidation was also determined. Mitochondrial swelling was evaluated as the change in absorbance under succinate-energized conditions. Cytochrome c release and mitochondrial ATPase activity were assessed. The results showed that PCK and CoQ10 significantly scavenged DPPH and nitric oxide radicals in a concentration-dependent manner and demonstrated a better ferricreducing antioxidant potential. PCK exhibited a high DPPH radical scavenging ability with the lowest IC_{50} value of 54.05 µg/mL while CoQ10 exhibited higher reducing power with the IC_{50} value of 82.14 µg/mL.Both were also found to inhibit angiotensin-converting enzyme activity. In addition, PCK and CoQ10 significantly ($p<0.05$) prevented lipid peroxidation, modulated the opening of mitochondrial permeability transition (mPT) pores and caused no significant release of cytochrome c. However, CoQ10 showed a mild inductive effect on mPT pores at higher concentrations. PCK and CoQ10 also increased mitochondrial ATPase activity. The results of this study suggest that both PCK and CoQ10 may be helpful in the treatment of diseases such as neurological disorders where excessive apoptosis is associated with excessive tissue degradation.

Keywords: Coenzyme Q10, Potassium Polyacrylate, Antioxidants, Mitochondrial Permeability Transition, Apoptosis.

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1. Introduction

In addition to their primary metabolic role in oxidative phosphorylation, which is the process by which energy is produced, mitochondria also play a crucial role in the onset of apoptosis. Certain anticancer drugs have been reported to contribute significantly to the apoptotic process by altering mitochondrial processes, including the permeability transition. Increased matrix calcium typically originates from mitochondrial malfunction, which is mostly caused by abnormalities in mitochondrial genes (1). Overloading the mitochondria with calcium (Ca^{2+}) results in an excess of oxygen-derived free radicals being generated. This leads to the opening of the mega transition pore, or mitochondrial permeability transition (mPT) pore. Adenine nucleotide translocator (ANT) in the inner membrane, the voltage-dependent anion channel (VDAC) in the outer membrane, cyclophilin D (CypD) in the matrix, and numerous other molecules make up the complex structure of the mPT pore, a high conductance channel in the mitochondria (2). CypD is found in the mitochondrial matrix, and the mPT pore is generally closed. Variations in the expression of the anti-apoptotic protein B-cell lymphoma-2 (Bcl-2), ionic Ca^{2+}, pH, reactive oxygen species (ROS), and ADP/ATP levels are just a few of the many variables that delicately controls its opening (3). In mitochondria, a short-term opening of mPT pores has physiological significance. Long-term opening, on the other hand, causes mitochondrial depolarization, loss of mitochondrial membrane potential, conversion of the mitochondrial ATP synthase enzyme into phosphatase, oxidative stress, mitochondrial swelling, and release of apoptosis-promoting proteins such as cytochrome c into the cytosol (4), ultimately leading cell death. The mitochondrial permeability transition pore has been implicated in both diseases associated with excessive apoptosis, such as neurological diseases, and diseases associated with uncontrolled cell proliferation, such as cancer (5). The mitochondrial permeability transition pore has become a therapeutic target in a number of diseases (6). We have previously reported the pharmacological effects of numerous medicinal plants and bioactive compounds on the mitochondrial transition pore (7, 8). According to studies, several compounds can be used to delay or prevent the opening of permeability transition pores, which can always be used to avoid tissue deterioration (9). The search for medications that can induce the opening of the permeability transition pore and result in large amplitude swelling of the mitochondria is also ongoing in conditions where apoptosis is downregulated. Cell death has been shown to be induced by dietary components and secondary metabolites. Due to the presence of components that can act as inducers or modulators of the mitochondrial permeability transition pore, medicinal plants, and bioactive chemicals have become important in the pharmacological targeting of mitochondria (10). Potassium polyacrylate (PCK), a superabsorbent polymer of water and electrolytes is used in food processing, cosmetics, consumer products, and pharmaceuticals. The biological effects of PCK have been characterized after subchronic oral administration with no adverse histopathological changes (11). The activity of PCK in the prevention of blood pressure elevation in rats with renal failure on a high-sodium diet, and its inhibitory and pharmacological potential on angiotensin-converting enzyme associated with hypertension via in silico study have also been investigated (12). It has been proposed that the polyanionic structure of PCK allows it to chelate different metals. However, little is known about the antioxidant capacity of PCK and its effects on mitochondrial permeability transition pores. Coenzyme Q10, an established electron transporter of the respiratory chain in the mitochondria, moves electrons from complexes I and II to complex III during oxidative phosphorylation and energy production. In addition to its vital function, research has shown that CoQ10 acts as a comprehensive scavenger of free radicals, reducing cell death and protecting cells from a variety of stressful conditions, such as neurological diseases (13). One potentially effective therapeutic approach for the treatmeny of mitochondrial diseases is the pharmacological suppression of mPT pores (14). Researchers have long desired the ability to target mitochondria with specific molecules. Therefore, this study investigated the *in vitro* antioxidative potential of potassium polyacrylate and coenzyme Q10 and their effects on mitochondrial permeability transition pores.

2. Materials and Methods

2.1. Chemicals and Reagents

Coenzyme Q10, potassium polyacrylate, HEPES, Adenosine triphosphate (ATP), Mannitol, Rotenone, Sucrose, Spermine, EGTA, TCA, Sodium Dodecyl Sulfate (SDS), Tris-HCl, Bovine serum albumin (BSA), Folin-Cioclateau, Sodium succinate, were supplied from Sigma. NH4Molybdate, L-ascorbate, $CaCl_2$, Na_2CO_3, $CuSO_4.5H_2O$, Na_2PO_4, NaOH, KCl, Na^+K^+ tartrate, were of analytical grade and significance.

2.2. Methods

The method of Braca et al. (15) was used to determine the DPPH radical scavenging activity of CoQ10 and PCK. The ferric reducing antioxidant power (FRAP) of CoQ10 and PCK was determined by the method of Oyaizu (16) with slight modifications. The nitric oxide radical scavenging capacities of CoQ10 and PCK were measured by the Griess reaction (17). The angiotensin-converting enzyme (ACE) inhibitory activity of CoQ10 and PCK were determined by the method of Cushman and Cheung, (18) with slight modifications. Lipid peroxidation was assessed by the method of Ruberto et al. (19). Hepatic mitochondria were isolated as described by Johnson and Lardy (20). Mitochondrial protein was determined as previously described using bovine serum albumin (BSA) as the standard (21). Mitochondrial swelling was evaluated by the method of Lapidus and Sokolove (22). Cytochrome c release from isolated mitochondria was d determined spectrophotometrically according to Appaix et al. (23).

Mitochondrial F_0F_1 ATPase activity was measured according to the method of Lard and Wellman, (24).

2.3. Statistical Analysis

Data were analyzed using one-way ANOVA with Dunnett's multiple comparison post hoc test. Data were analyzed using GraphPad Prism version 5.0. All data are presented as mean ± SEM. The significance level was set at $p<0.05$.

3. Results

3.1 *In Vitro* Antioxidant and Angiotensin-Converting Enzyme Inhibitory Activities

The *in vitro* antioxidant activities of potassium polyacrylate (PCK) and coenzyme Q10 (CoQ10) are shown in (Figure 1). The PCK and CoQ10 have antioxidant properties. They scavenged DDPH radicals in a concentration-dependent manner with IC_{50} values of 54.05 µg/mL and 54.68 µg/mL for PCK and CoQ10 respectively (Table 1). PCK showed a high radical scavenging ability with the lowest IC_{50} value. The results showed that both PCK and CoQ10 scavenged the DPPH radical more than the standard, ascorbic acid (Figure 1A). The results of the reducing potential of PCK and CoQ10 are shown in Figure 1B. The reduction of Fe^{3+} to Fe^{2+} in the presence of PCK and CoQ10 was used to evaluate the reductive capability of the compounds. CoQ10 exhibited higher reducing power with the lowest IC_{50} value 82.14 µg/mL when compared to PCK 88.71 µg/mL and the standard, ascorbic acid 83.08 µg/mL. The difference in the reducing power between CoQ10 and PCK is significant at ($P < 0.05$). The results showed that PCK and CoQ10 exhibited nitric oxide radical scavenging activity. It was observed from the results that the standard, butylated hydroxytoluene had greater NO radical scavenging activity at all the concentrations with the lowest IC_{50} value of 164.04 µg/mL which is significant at ($P < 0.05$) when compared to PCK and CoQ10 (Fig. 1C). The angiotensin-converting enzyme (ACE) inhibitory activity is shown in Figure 1D. The results indicated that both the PCK and CoQ10 exhibited ACE inhibitory potential. However, the percentage ACE inhibitory potential of the reference standard, Lisinopril was significantly ($P < 0.05$) higher than that of PCK and CoQ10 at all concentrations tested with the lowest IC_{50} values of 132.21 µg/mL.

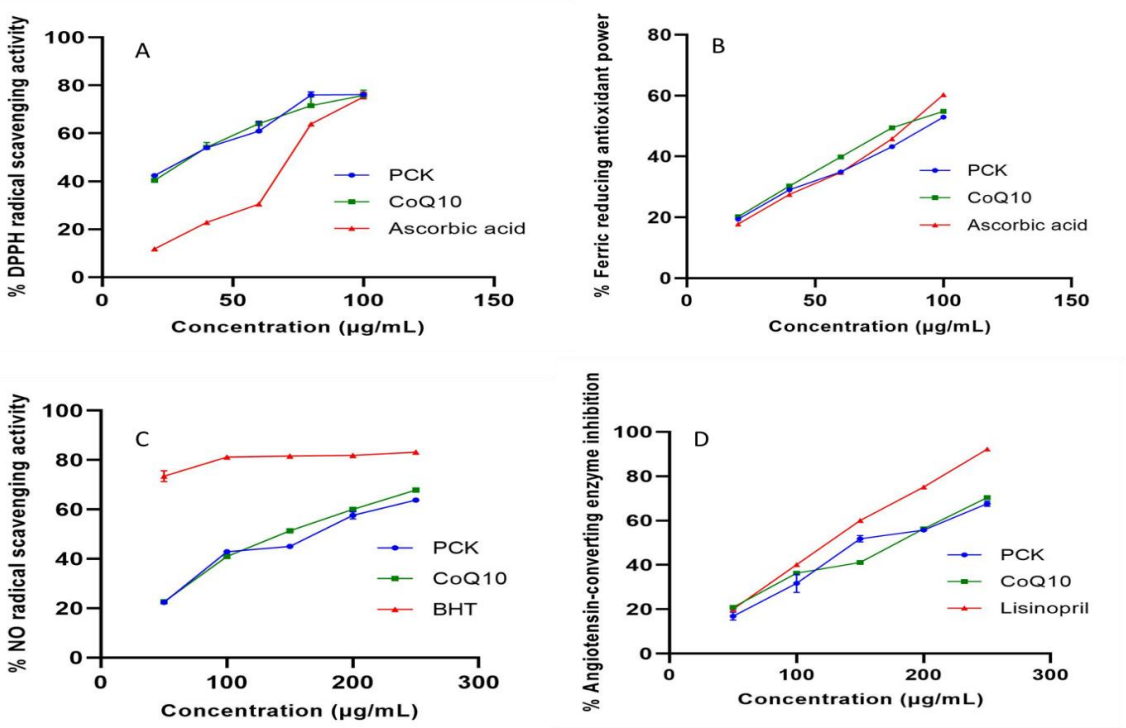

Figure 1. *In vitro* antioxidant and angiotensin-converting enzyme inhibitory activities of potassium polyacrylate and Coenzyme Q10. Data = Mean ± SEM; n = 3. BTH = Butylated hydroxytoluene

Table 1. IC_{50} values of PCK and CoQ10 in the *in vitro* antioxidant activities and angiotensin-converting enzyme inhibitory activities.

IC_{50} (µg/mL)	DPPH	FRAP	NO	ACE
PCK	54.05	88.71	173.43	172.65
CoQ10	54.68	82.14	164.04	173.13
Ascorbic acid	70.49	83.08	-	-
Butylated hydroxytoluene	-	-	112.31	
Lisinopril	-	-	-	132.21

DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; NO: nitric oxide; ACE: angiotensin-converting enzyme

3.2. Assessment of Lipid Peroxidation

The results of the percentage inhibition of lipid peroxidation are shown in Figure 2. PCK and CoQ10 inhibited the lipid peroxidation. It was observed that the percentage inhibition of lipid peroxidation of both the PCK and CoQ10 were concentration dependent.

3.3. Assessment of Mitochondrial Integrity

Figure 3 describes the assessment of calcium and spermine on mitochondrial permeability transition pores in rat liver mitochondria. The results obtained showed the integrity (intactness) of the isolated mitochondria as there was no significant change (decrease) in the absorbance of the mitochondria within the 12 minutes recorded at 540 nm in the absence of exogenous calcium. This indicated that there was no induction of mPT pore opening stimulated by succinate. A large amplitude of mitochondria swelling as shown by a significant drastic decrease in the absorbance at 540 nm, was observed in the presence of exogenous calcium (0.274 ± 0.005) with an induction fold of 4.72 (Table 2). The opening of the mPT pore was significantly inhibited by spermine by 75.18 % (Table 3).

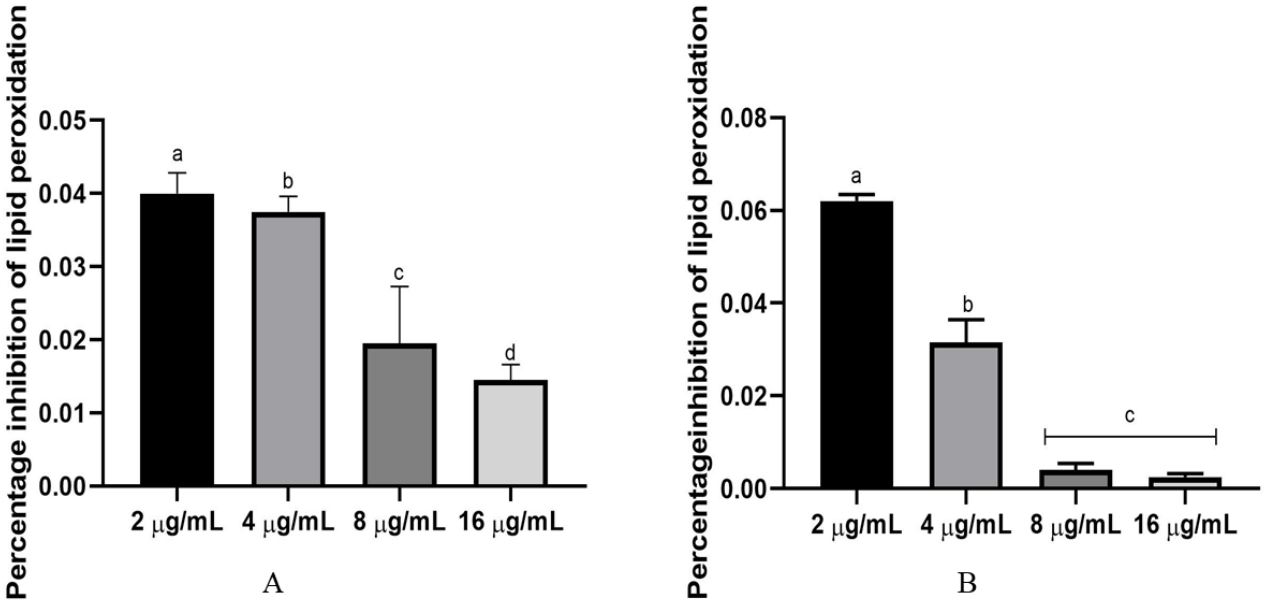

Figure 2: Percentage of lipid peroxidation inhibitory activities of (A) potassium polyacrylate and (B) coenzyme Q10.
Data = Mean ± SEM; n = 3. Values with different letters above the bars for a given concentration are significantly ($p < 0.05$) different from each other.

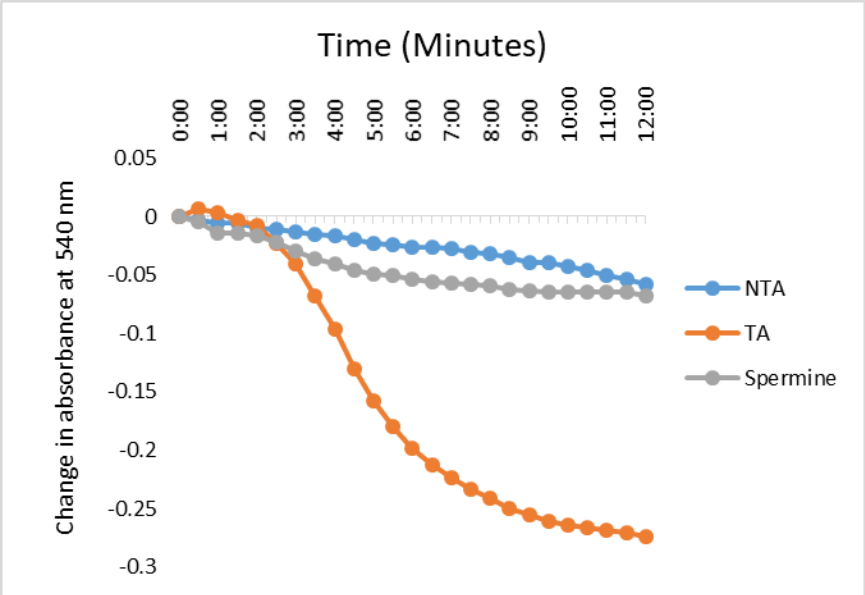

Figure 3: Calcium-induced opening of the mitochondrial membrane permeability transition pore of normal rat liver and the reversal effect of spermine.
NTA= No Triggering Agent; TA= Triggering Agent; Spermine = Inhibitor.

Table 2: Percentage induction of mitochondrial permeability transition pores by calcium.

Groups	Change in Absorbance	Induction fold	% Induction
NTA	0.058 ± 0.002	1.0	-
TA	0.274 ± 0.005	4.72	78.83

Results are expressed as the mean of three determinations ± standard error of the mean (SEM).
NTA: No triggering agent (- Ca^{2+}); TA: Triggering agent (+ Ca^{2+})

Table 3: Change in absorbance at 540nm of the effect of PCK and CoQ10 in the absence of exogenous calcium.

Groups	Change in Absorbance	Inhibition fold	% Inhibition
NTA	0.058 ± 0.002	1.0	-
Spermine	0.068 ± 0.004	4.03	75.18
16 µg/mL PCK	0.098 ± 0.005	2.80	64.23
32 µg/mL PCK	0.080 ± 0.003	3.43	70.80
48 µg/mL PCK	0.099 ± 0.002	2.77	63.87
64 µg/mL PCK	0.097 ± 0.003	2.82	64.60
80 µg/mL PCK	0.087 ± 0.001	3.15	68.25
16 µg/mL CoQ10	0.103 ± 0.007	2.66	62.41
32 µg/mL CoQ10	0.079 ± 0.005	3.47	71.17
48 µg/mL CoQ10	0.100 ± 0.002	2.74	63.50
64 µg/mL CoQ10	0.258 ± 0.006	1.06	58.39
80 µg/mL CoQ10	0.194 ± 0.004	1.41	29.20

Results are expressed as the mean of three determinations ± standard error of the mean (SEM).
NTA: No triggering agent (- Ca^{2+}); Spermine: Reference Inhibitor

3.4. Effect of PCK and Coq10 On the Mitochondrial Permeability Transition Pore in the Absence and Presence of Calcium

The effect of different concentrations (16, 32, 48, 64, 80 µg/mL) of PCK and CoQ10 on the mPT pore in the absence and presence of calcium is shown in Figures 4 and 5, respectively. The results showed that in the absence of the triggering agent, calcium, PCK significantly inhibited the mPT pore opening at all concentrations (Fig. 4A). The percentage inhibition of the pore opening by PCK in the absence of calcium at (16, 32, 48, 64, 80 µg/mL) are 64.23%, 70.80%, 63.87%, 64.60%, and 68.25% ,respectively (Table 3). The inhibition of mPT pore by PCK is concentration dependent at (48, 64, and 80 µg/mL). CoQ10 inhibited pore opening in the same manner but showed a mild inductive effect at (64 and 80 µg/mL) (Figure 4B). In the presence of calcium, both PCK and CoQ10 potentiated the opening of mPT pore opening at all concentrations. It was also observed that in the presence of calcium, the lowest concentration (16 µg/mL) for both PCK and CoQ10 significantly induced pore opening compared to higher concentrations (Figures 5A & B).

3.5. Assessment of Cytochrome C Release and Mitochondrial ATPase

Using the mitochondria as the lipid-rich media, the effect of PCK and CoQ10 on cytochrome c release and mitochondrial ATPase activity was evaluated. The results showed that there was no cytochrome c release in the absence of calcium (Figures 6A & B). However, a minimal amount of cytochrome c release was observed at 64 and 80 µg/mL in CoQ10, c consistent with the mild inductive effects on mPT pore opening observed at these concentrations. In the presence of calcium, a significant ($p < 0.05$) increase in the concentration of cytochrome c released into the reaction medium was observed for both PCK and CoQ10. The effect of PCK and CoQ10 on mitochondrial ATPase is presented in Figures 7A & B. The results showed that different concentrations of both the PCK and CoQ10 increased the activity of mitochondrial ATPase when compared with the control. However, the increase in mitochondrial ATPase activity by PCK at all concentrations is not significantly ($p < 0.05$) different from that by calcium. It was observed that CoQ10 had significantly $P < 0.05$ higher ATPase activity at the highest concentration (80 µg/mL). From the results, it was also observed that the uncoupler (2, 4 -dinitrophenol) at 25µM increased the mitochondrial ATPase activity.

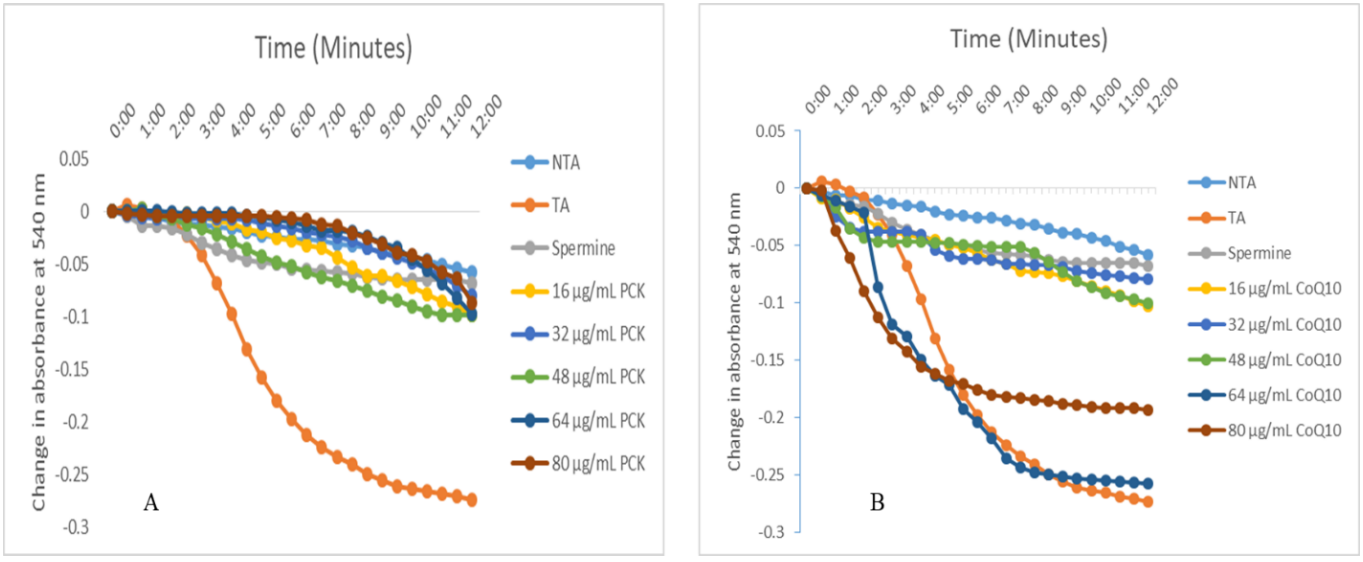

Figure 4: Varying concentrations of (A) potassium polyacrylate and (B) coenzyme Q10 on the mitochondrial membrane permeability transition pore of rat liver in the absence of exogenous calcium.
NTA= No Triggering Agent; TA= Triggering Agent; Spermine = Inhibitor.

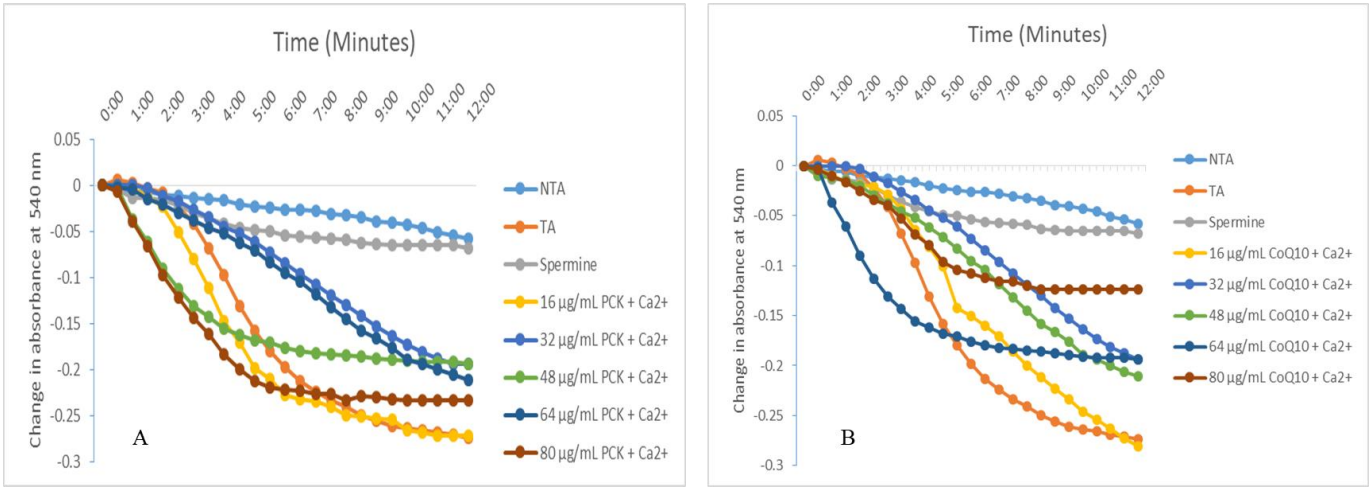

Figure 5: Varying concentrations of (A) potassium polyacrylate and (B) coenzyme Q10 on the mitochondrial membrane permeability transition pore of rat liver in the presence of exogenous calcium.
NTA= No Triggering Agent; TA= Triggering Agent; Spermine = Inhibitor.

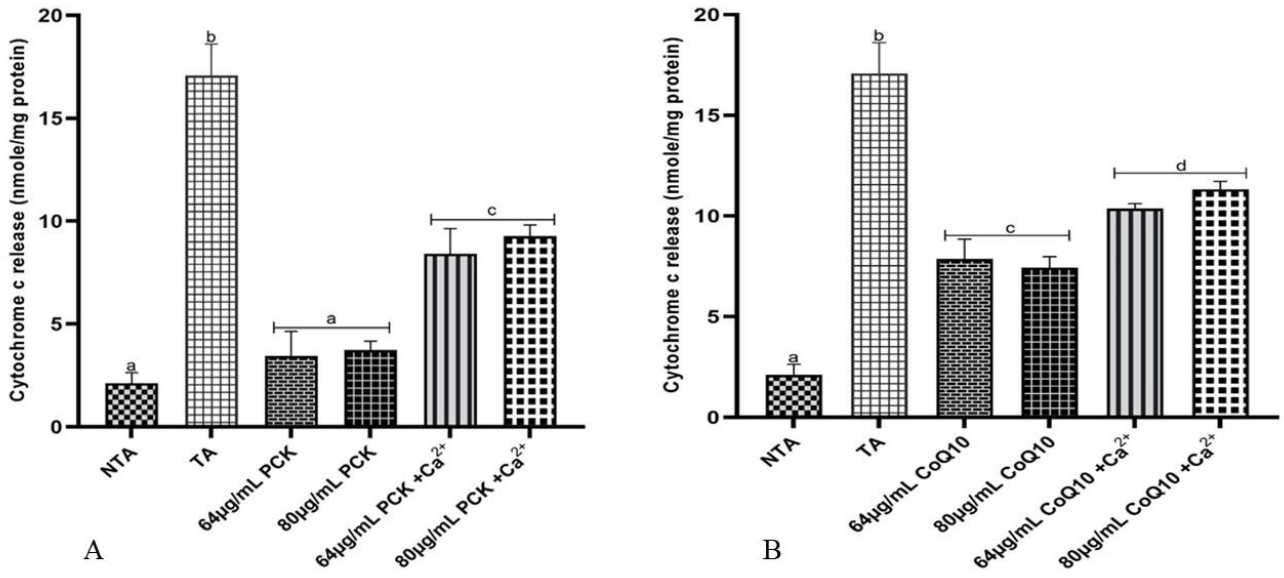

Figure 6: Effect of different concentrations of (A) potassium polyacrylate and (B) coenzyme Q10 on cytochrome c release from rat liver mitochondria.
Data = Mean ± SEM; n = 3. Values with different letters above the bars for a given concentration are significantly ($p < 0.05$) different from each other.

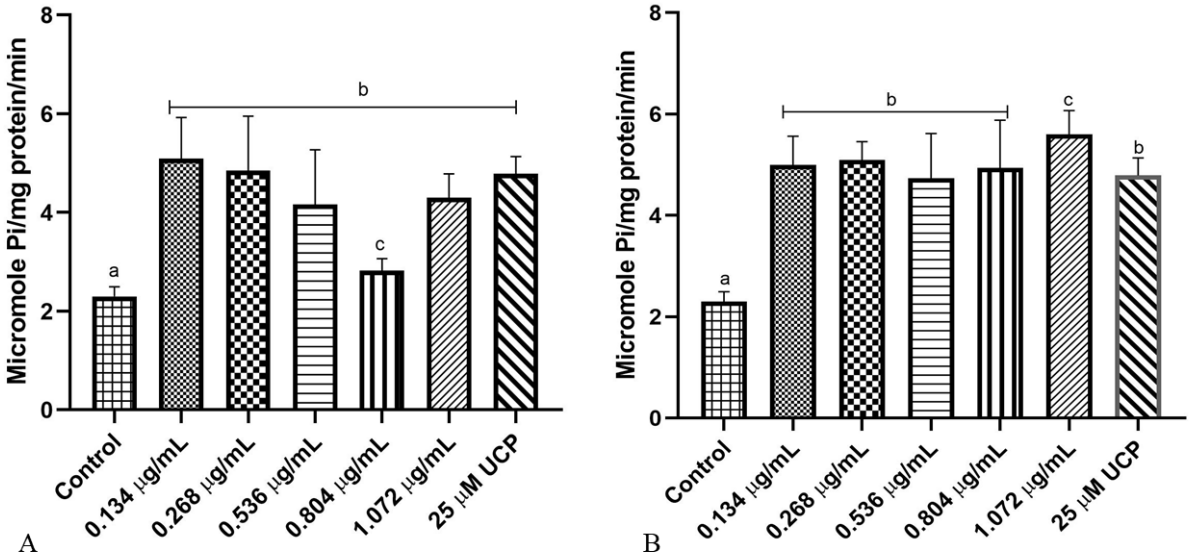

Figure 7: Mitochondrial ATPase activities of (A) potassium polyacrylate and (B) coenzyme Q10.
Data = Mean ± SEM; n = 3. Values with different letters above the bars for a given concentration are significantly ($p < 0.05$) different from each other.

4. Discussion

A complex, large conductance channel known as the mitochondrial permeability transition pore is crucial for the induction of apoptosis. The rupture of the mitochondrial transmembrane electrochemical gradient and the subsequent oligomerization of apoptotic bcl-2 family members is caused by the opening of the mPT pore. Oxidative stress and mitochondrial dysfunction are the main factors that cause the development of mitochondrial permeability transition pores and cell death. Studies have shown that mitochondrial damage can result from oxidative stress caused by increased production of reactive oxygen species (25). Previous publications suggest that a variety of processes, such as scavenging of reactive oxygen species and initiating or inhibiting the mitochondrial-mediated pathway of apoptosis, are used by pure compounds and secondary metabolites to achieve their therapeutic effects. The free radical scavenging capacity of diverse samples has often been evaluated using the free radical compound DPPH. According to this study we understood that both the potassium polyacrylate (PCK) and coenzyme Q10 (CoQ10) exhibited DPPH radical scavenging activities. The degree of DPPH reduction or decolorization by PCK and CoQ10 is concentration dependent. The DPPH radicalscavenging abilities of both PCK and CoQ10 were found to be significantly higher than the reference standard, ascorbic acid. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (26). The results showed that PCK and CoQ10 have greater ferric reducing capabilities. Increased absorbance of the reaction mixture correlates with greater reducing power. Studies have shown that the polyanionic nature of PCK allows it to chelate various metals (11). The observation from this study is also consistent with the previous work of Navas et al. (27), who reported the free radicalscavenging ability of CoQ10. Nitric oxide, an important cell mediator controls a number of processes in biological systems. It has anti-inflammatory, vasodilatory, proliferative, and cardiovascular effects, among others. Excessive levels of nitric oxide can have adverse effects on organisms. Data from this study showed that PCK and CoQ10 scavenged nitric oxide radicals, suggesting that which suggests that PCK and CoQ10 may prevent the harmful effects of nitric oxide radicals. Angiotensin-converting enzyme, a critical component of the renin-angiotensin system mediates a variety of systemic and local cardiovascular effects by preventing the conversion of angiotensin I to angiotensin II, a vasoconstrictor. The results of this study indicated that PCK and CoQ10 inhibited angiotensin-converting enzymes in a concentration-dependent manner. Our previous study also showed that PCK and CoQ10 have favorable binding affinities to ACE and interact with the S1 and S2 binding pocket of ACE via in silico analysis (12). In the cell membranes, polyunsaturated fatty acids undergo an oxidative modification known as lipid peroxidation, which produces a number of breakdown products. Through the peroxidation of membrane lipids, an increase in reactive oxygen species has been shown to cause membrane damage (28). The ability of PCK and CoQ10 to inhibit lipid peroxidation, and scavenge DPPH and nitric oxide radicals is indicative of their antioxidant properties, thereby presenting them as potential compounds for treating/preventing conditions caused by oxidative stress. It is alsopossible that PCK and CoQ10 protect against radical-induced severe cellular damage, preserving the physiochemical characteristics of membrane bilayers. Inhibition of the growth of lipid peroxidation is one of known functions of ubiquinol. The ability of CoQ10 to recycle other antioxidants such as vitamin C

and vitamin E is also a factor in the antioxidant capabilities of CoQ10 (29). CoQ10 is not only widely distributed in the lipid milieu of cellular membranes but also in plasma lipoproteins, where it functions as an antioxidant defense. One of the possible explanations for the antiatherogenic effects of CoQ10 is its involvement in the antioxidant mechanisms that protect low-density lipoproteins from oxidation. Due to the loss of mitochondrial membrane potential and an increase in calcium concentration, uncontrolled activation of the mPT pore inevitably leads to programmed cell death or necrosis. While the use of CoQ10 for the treatment of both mitochondrial diseases and neurodegenerative diseases has attracted much interest (13), the effect of PCK on the mitochondrial permeability transition pore has not been described. From the results, it was observed that there was no change in the absorbance of mitochondria under succinate-energized situations without calcium, indicating the integrity of the isolated mitochondria. However, a large amplitude swelling was observed in the presence of calcium, which was significantly reversed by spermine. PCK modulated the opening of mPT pores in the absence of calcium at all concentrations tested. CoQ10 also modulated the pore opening at the lower concentrations but caused a mild inductive effect at the higher concentrations. The ability of several ubiquinone analogs to influence the open-closed transition of mPT pores in isolated mitochondria has been reported previously (30). These compounds are thought to act through a shared PTP-binding site rather than through oxidation-reduction processes. In the presence of calcium, both PCK and CoQ10 potentiated mPT pore opening at all concentrations. Therefore, we propose that mPT pore opening in the presence of calcium doesn't serve an independent purpose, but it is most likely related to the assembly of multicomplex units, and its modulation reflects changes in mitochondrial activity. According to physiological and biochemical research, CoQ10 may also have a direct inhibitory effect on the opening of the mPT pore during the reperfusion of ischemic hearts, which would explain its cardioprotective and apoptosis-preventive effects (31, 32). An increase in the amount of inorganic phosphate in the cytosol and the release of the enzyme cytochrome c are two indicators of the onset of apoptosis, a basic biological event in eukaryotic cells that is necessary to maintain tissue homeostasis. There was a significant increase in mPT pore opening as evidenced by the increase in cytochrome c release observed during the study in the presence of calcium. The results showed that PCK did not contribute to the release of cytochrome c. The dynamics of ATP synthase are altered by the depolarization of the mitochondrial membrane potential resulting in less ATP synthesis. This accelerates the process of cellular apoptosis, which leads to energy depletion. The results showed that the different concentrations of both the PCK and CoQ10 increased the activity of mitochondrial ATPase compared to the control. However, the increase in the mitochondrial ATPase activity by PCK at all concentrations is not significantly different from calcium. It was also observed that CoQ10 had significantly higher ATPase activity at the highest concentration. The results also showed that the uncoupler (2, 4-dinitrophenol) increased mitochondrial ATPase activity. Several neurodegenerative diseases have been linked to defects in the mitochondrial respiratory chain and oxidative phosphorylation. CoQ10 administration has been reported to dramatically improve ATP production in the cardiac and skeletal muscles of patients with various neurodegenerative diseases. In this study, we demonstrated for the first time that potassium polyacrylate (PCK) has antioxidant potential. Moreover, PCK and CoQ10 prevented lipid peroxidation, modulated the opening of mitochondrial permeability transition pore, and didn't generate a significant release of cytochrome c. PCK and CoQ10 also increased mitochondrial ATPase activity. The results of this study suggest that both PCK and CoQ10 may be helpful in the treatment of diseases such as neurological disorders, where excessive apoptosis is accompanied by excessive tissue degradation.

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Authors' Contribution
Conceptualization, Investigation, Data curation, Writing- Review and Editing: AOA.
Investigation, Data curation, Review and editing: JOO.
Data curation, Review and editing: DJP.
Investigation, Data curation, Review and editing: JOA
Data curation, Review and editing: MAR.
Conceptualization, Data curation, Review and editing: NHG.

Ethics
Ethical approval for the use of animals is not required due to the limited number of animals used. All procedures in this study conformed to the tenets of animal research as recommended by the Declaration of Helsinki and the National Institute of Health.

Conflict of Interest
The authors declare that they have no conflict of interests.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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