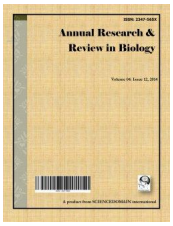

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Evaluation of the Effects of Fractions of *Jatropha curcas (Linn)* Leaves on Mitochondrial Permeability Transition in Rat Liver

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOO designed the study. Author OTO wrote the protocol and edited the first draft of the manuscript written by author WRO. Authors WRO and OFA performed the statistical analysis and the analyses of the study was managed by all authors. Authors OTO and WRO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Modulation of the opening of mitochondrial Permeability Transition (mPT) pore is an important pharmacological target in drug design because the release of cytochrome C upon the opening of the pore is sine *qua non* for mitochondrial-mediated apoptosis to take place. *Jatropha curcas* has been shown to inhibit the growth of tumorigenic cells in different cancer cells. It is not known whether the intrinsic pathway of apoptosis is implicated in the mechanism of action of *J. curcas* as an anti-tumour agent. This study evaluated the effects of varying concentrations of the methanol extract of *Jatropha curcas* and its fractions on mPT pore and cytochrome C release.
Methodology: Mitochondrial permeability transition, mitochondrial lipid peroxidation, mitochondrial lipid peroxidation, cytochrome C release were evaluated spectrophotometrically.
Results: Chloroform fraction of *J. curcas* (CFJC) significantly induced pore opening at 10, 30, 50, 70 and 90 µg/mL by 2.2, 3.5, 8.5, 11.0 and 11.7 folds, respectively in the absence of calcium when

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compared with other fractions. In contrast calcium -induced pore opening was inhibited by all the fractions. Interestingly, all the fractions also inhibited Fe^{2+}-induced lipid peroxidation in a concentration-dependent manner. Mitochondrial ATPase activity was enhanced to varying extents by these fractions with CFJC having the highest stimulatory effects. The concentration of cytochrome C released from mitochondria exposed to CFJC at 10, 30, 50, 70 and 90µg/mL were significantly ($p < 0.05$) elevated by 5, 10, 12.5, 14.2 and 17 folds, respectively when compared with control.

Conclusion: These findings suggest that CFJC, the most potent fraction may contain certain bioactive agent(s) that modulates mPT pore opening and possibly induces mitochondrial-mediated apoptosis via release of cytochrome C and reduction of intracellular ATP levels. *Jatropha curcas* may therefore prove useful in drug development in diseases characterized by insufficient apoptosis.

Keywords: Mitochondria; apoptosis; Jatropha curcas; permeability transition; cytochrome C.

1. INTRODUCTION

Cell death is an essential part of the normal development and maturation cycle [1]. A homeostatic balance between the rates of cell proliferation and cell death is critical for maintenance of physiological processes. Alteration or defiance against these natural death mechanisms can lead to diseases such as AIDS, diabetes mellitus, neurodegenerative diseases and cancer [2]. Evidence abound that most of these diseases are reactive oxygen species mediated [3]. The mechanisms of apoptosis are highly complex and sophisticated, involving an energy-dependent cascade of molecular events. Several studies have indicated that there are two main apoptotic pathways; the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway [4,5]. Mitochondria are cellular organelles intimately involved in energy production and are essential for cellular life. However, research has shown that these organelles play an important role in cell death which occurs when their membranes become permeabilized [6,7]. Permeabilization of the outer mitochondrial membrane has been recognized as a major event in the induction of the mitochondrial pathway of apoptosis because opening of the pore causes the release of a range of pro-apoptotic proteins from the intermembrane space into the cytosol [6,8,9]. These include cytochrome C, [9] AIF [10], Smac/Diablo [11], the serine protease HtrA2/Omi and endonuclease G [12]. It is well established that release of cytochrome C into the cytosol triggers caspase activation and ultimately apoptosis [9]. The nature of the pore through which these proteins are released is still uncertain and the identity of the proteins involved in its formation is highly controversial [13,14]. However, certain bioactive agents including betulinic acid have been shown to modulate the opening of the mPT pore [15,16]. *Jatropha curcas* commonly called *physic* /barbados nut is a small tree or shrub belonging to family *Euphorbiaceae* [17]. It originated from Central America but has spread to other tropical and subtropical countries and mainly grows in Asia and Africa. It is a tall bush/shrub or small tree that can grow up to 6 meters tall. The *Jatropha* plants show different plant architecture, ranging from a main stem with no or few branches to a plant that is branched from below. Studies have shown that the leaves of *Jatropha curcas* contain tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides [18].

In tropical Africa, *J. curcas* preparations are used for the treatment of diverse ailments including dysentery, fever, rheumatism, jaundice and malaria [19,20,21]. Extracts of the multipurpose plant have been reported to possess anti-inflammatory [22], anti-microbial [23], anti-parasitic [24], and anti-viral [25]. Specifically, the methanolic extract of *J. curcas* leaves showed anti-metastatic activity [26] while isoamericanol A from *Jatropha curcas* seeds inhibited growth of MCF-7 human breast cancer cells via cell cycle arrest [27]. It is not known whether the intrinsic pathway of apoptosis, a phenomenon that is downregulated in most cancer cells is implicated in the mechanism of action of *J. curcas* against transformed cells. This study was therefore designed to determine the modulatory effect of various fractions of the extract of *Jatropha curcas* leaves on mitochondrial permeability transition via the release of cytochrome C.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

Cytochrome C, Mannitol, sucrose, N-2-hydroxy-ethyl-pipe-arizine-N-2-ethanesulfonic acid (HEPES), rotenone, spermine, Folin-Ciocalteu reagent, Bovine Serum Albumin (BSA), and all

other reagents were of highest purity grade and were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA).

2.2 Experimental Animals and Ethical Consideration

Forty- five male Wistar strain albino rats weighing between 80 g-100 g were obtained from the Pre-Clinical Animal House, University of Ibadan, Ibadan, Nigeria. The animals were allowed to acclimatize for 14 days in cages in the Animal House of the Department of Biochemistry, University of Ibadan, had access to water and chow *ad libitum* and were kept under standard conditions of temperature and humidity.

2.3 Preparation of Plant Material

Fresh leaves of *J. curcas* were obtained from a local farmland in Ibadan, Oyo State, Nigeria. Botanical identification and authentication was carried out at the Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Nigeria. The fresh plant was separated from extraneous materials and washed. The leaves were air dried for about two weeks and then blended to powder. Air-dried, powdered leaves of *J. curcas* were soaked with sufficient methanol in all- glass jars at room temperature for 72 hrs. The filtrate was concentrated under reduced pressure using a rotary evaporator to obtain the crude methanol extract of *J. curcas* (MEJC).

2.4 Partitioning of Crude Methanol Extract of *Jatropha curcas* (MEJC) Using Vacuum Liquid Chromato-graphy

Crude methanol extract of *J. curcas* (10 g) was preabsorbed with silica gel 60 (10 g) (0.040–0.063 mm, MERCK). The mixture was air-dried to obtain a powdery form. A sintered funnel for Vacuum Liquid Chromatography (VLC) was packed with 20 g Silica gel (Hopkins and Williams, England). Solvents were added in order of increasing polarity and n-hexane, chloroform, ethylacetate and methanol fractions were obtained in this order. All eluted fractions were concentrated to dryness under pressure using rotary evaporator at 40°C to obtain the n-hexane (HFJC). Chloroform (CFJC), ethylacetate (EFJC) and the methanol (MFJC) fractions of the crude extract. They were later stored in glass sample bottles and kept in the refrigerator until use.

2.5 Isolation of Rat Liver Mitochondria

Rat liver mitochondria were isolated essentially according to the method of Johnson and Lardy [28] and as modified by Olorunsogo et al. [29]. The animals were sacrificed by cervical dislocation and the livers excised and trimmed to wash excess tissue. The livers were then weighed, washed with homogenizing buffer (210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES-KOH, pH 7.4 and 1 mM EGTA), and homogenized as a 10% suspension in ice-cold buffer using a Porter Elvehjem glass homogenizer. The resulting homogenate was centrifuged in an MSE refrigerated centrifuge at 2300 rpm for 5 mins to remove the nuclear debris. This was done twice and the supernatant obtained was centrifuged at 13,000 rpm for 10 mins to obtain the mitochondrial pellet. The supernatant was discarded while the pellet was washed twice with the washing buffer (210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES-KOH, pH 7.4, 0.5% BSA) at 12,000 rpm for 10 mins. The mitochondria obtained were immediately resuspended in an appropriate volume of MSH buffer (210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES-KOH, pH 7.4) and immediately dispensed into Eppendorf tubes and kept on ice.

2.6 Mitochondrial Swelling Assay

Mitochondrial permeability transition was monitored by measuring changes in absorbance of mitochondrial suspension in the presence or absence of calcium (the triggering agent) in a T70 UV/visible spectrophotometer essentially according to the method of Lapidus and Sokolove [30]. Mitochondria (0.4 mg protein/mL) were pre-incubated in the presence of 0.8 µM rotenone in a medium containing 210 mM mannitol, 70 mM sucrose and 5 mM HEPES-KOH (pH 7.4) for 3 mins at 27°C prior to the addition of 120 µM $CaCl_2$. Thirty seconds later, 5 mM succinate was added and mitochondrial permeability transition quantified at 540 nm for 12 mins at 30 secs interval. To determine the integrity of the mitochondrial membrane, 4 mM spermine was immediately added following the addition of rotenone and just before the addition of mitochondrial fraction.

2.7 Determination of Mitochondrial Protein

Mitochondrial protein concentration was determined according to the method of Lowry et

al. [31] using Bovine Serum Albumin as standard.

2.8 Mitochondrial ATPase Activity

Mitochondrial ATPase activity was estimated by a modification of the method of Lardy and Wellman [32] as modified by Olorunsogo and Malomo [33]. Each reaction vessel contained 65 mM Tris-HCl (pH 7.4) 50 mM KCl, 1 mM ATP and 250mM sucrose in a final volume of 2 mL. The reaction which was initiated by the addition of mitochondrial fraction (0.5 mg protein/mL) was allowed to proceed for at 30°C with constant shaking for 30 mins. The reaction was quenched by the addition of ice-cold 10% trichloroacetic acid. The precipitated protein was separated by centrifugation and the phosphate content of the supernatant was promptly estimated by a standard colorimetric method. The blank contained all components of the basic incubation medium including ATP but devoid of mitochondria while 2, 4-Dinitrophenol (2, 4-DNP) was used as a standard uncoupling agent.

2.9 Estimation of Inorganic Phosphate Released

The concentration of inorganic phosphate released following the hydrolysis of ATP was determined according to the method described by Bassir (34) and as modified by Olorunsogo and Bababunmi (27). Briefly, 1.25% ammonium molybdate (1 mL) and 9% freshly prepared solution of ascorbic acid (1 mL) were added to 1 mL of the reaction mixture and allowed to stand for 30 minutes and absorbance was read at 680 nm.

2.10 Estimation of Mitochondrial Lipid Peroxidation

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using mitochondria as lipid rich media [35]. Mitochondria (1 mg/mL protein) and varying concentrations (100 µg-800 µg/mL) of fraction were added to each test tube and made up to 1 mL with distilled water; 0.05 mL of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and the mixture incubated for 30 mins. Then, 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added and the resulting mixture was vortexed and then heated at 95°C for 60 mins. After cooling, butan-1-ol (3 mL) were added to each tube and centrifuged at 3000 rpm for 10 min. the absorbance of the organic upper layer was measured at 532 nm. Percentage inhibition of lipid peroxidation by the extract was calculated as [AC-AE/AC] × 100. Where AC is the absorbance value of the fully oxidized control and AE is the absorbance in the presence of extract/fraction.

2.11 Assay for Cytochrome C Release

The quantitative determination of cytochrome C released from isolated mitochondria was performed by measuring the Soret (γ) peak for cytochrome C at 414 nm (ε = 100 $mM^{-1}cm^{-1}$), according to method of Appaix et al. [36]. Mitochondria (1 mg protein/mL) were pre-incubated in the presence of 0.8 µM rotenone in a medium containing 210 mM mannitol, 70 mM sucrose and 5 mM HEPES-KOH (pH 7.4) for 30 mins at 30°C in the presence of different concentrations of the fractions, using 24 mM calcium as the standard (TA). After the incubation, the mixture was centrifuged at 15,000 rpm for 10 mins. The optical density of the supernatant was measured at 414 nm which is the soret (γ) peak for cytochrome C.

2.12 Statistical Analysis

Statistical analysis was performed with Graph pad (version 5) using one-way analysis of variance (ANOVA). Level of significance was set at $p < 0.05$. All the results were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Results

There was no significant change in the volume of intact mitochondria respiring on succinate in the presence of rotenone over a period of twelve minutes (Fig. 1). The data presented shows that addition of calcium caused a highly significant increase in mPT pore opening and this was reversed upon addition of spermine, thus indicting that mitochondrial were intact *ab initio* and suitable for use.

Fig. 2 shows the effects of various concentrations of MEJC on mPT pore in the absence of calcium. From the results, 10, 30, 50, 70 and 90 µg/mL induced pore opening by 0.3, 1.1, 3.4, 14.9 and 16.8 folds, respectively, in a concentration-dependent manner when compared to control. Maximum induction (16.8 folds) was observed at 90 µg/mL and was higher than that obtained for calcium (14.6 folds), a standard triggering agent.

Fig. 3 depicts the effects of various concentrations of MEJC on mPT pore in the presence of calcium. In the presence of calcium, addition of different concentrations of MEJC to mitochondria resulted in inhibition of calcium-induced opening of the pore in a concentration-dependent manner. Specifically, percentage inhibition of 9.8, 10.9, 11.6, 12.8 and 15.5% was obtained at 10, 30, 50, 70 and 90 µg/mL MEJC, while spermine , a standard pore inhibitor gave 88.4% .

The effects of various concentrations of Chloroform Fraction of *Jatropha curcas* (CFJC) on the mPT in the absence of calcium are shown in Fig. 4. Similarly, varying concentrations of CFJC caused a significant induction of pore opening by 2.2, 3.5, 8.5, 11.0 and 11.7 folds, at 10, 30, 50, 70 and 90 µg/mL, respectively. However, the inductive effect (11.7 folds) at the highest concentration (90 µg/mL) was lower than that obtained on the addition of calcium (15.7 folds).

The effects of the exposure of varying concentrations of CFJC on isolated mitochondria in the presence of calcium are presented in Fig. 5. The results show that calcium-induced opening was inhibited in a concentration-dependent manner ($p<0.05$). In this regard, CFJC at 10, 30, 50, 70 and 90 µg/mL inhibited calcium-induced opening of the pore by 27.6, 28.9, 34.4, 47.6 and 51.2%, respectively while 88.8% was obtained for spermine, the standard inhibitor of pore opening.

Figs. 6 and 7 show the effects of Ethylacetate Fraction (EFJC) and Methanol Fraction (MFJC) of *Jatropha curcas* on mPT pore in the absence of calcium. At lower concentrations (10, 30 and 50 µg/mL), EFJC had no significant effect on mPT pore opening. However, at the higher concentrations (70 and 90 µg/mL), EFJC had significant inductive effect of 6.2 and 9.4 folds, respectively.

However, at all concentrations tested (10-90 µg/mL), MFJC had no effect whatsoever on mPT pore.

The effects of varying concentrations of EFJC and MFJC on mitochondrial permeability transition pore in the presence of calcium are shown in Figs. 8 and 9. Again, varying concentrations of EFJC and MFJC inhibited calcium-induced opening of the pore. At 10, 30, 50, 70 and 90 µg/mL, EFJC inhibited mPT pore opening by 40.7, 48.8, 59.1, 78.7 and 81.3%, respectively. Similarly, inhibition of the pore was achieved by MFJC at the same concentrations by 42.8, 45.2, 47.6, 48.8, and 51.5%, respectively. Spermine, a standard inhibitor, displayed 88.4% inhibition.

Fig. 10 shows the effects of varying concentrations of the fractions of crude MEJC on mitochondrial F_0F_1 ATPase activity. Mitochondrial ATPase activity was significantly enhanced by MEJC and CFJC in a concentration-dependent manner. Exposure of MEJC and CFJC to intact mitochondria (pH= 7.4) resulted in the release of 11.9 and 9.3 µmoles inorganic phosphate /mgprotein/min at the highest concentration (90 µg/mL) which was close to that of 2,4 - dinitrophenol, the standard uncoupler (14 µ moles Pi /mg protein/min). On the other hand, EFJC and MFJC had no significant effect on mitochondrial ATPase activity except at the highest concentration (90 µg/mL). Taken together, the results show that MEJC had the highest enhancement effects followed by CFJC while the effects showed by EFJC and MFJC were minimal.

Fig. 11 shows the inhibition of iron-induced lipid peroxidation by different fractions of *Jatropha curcas.* The results revealed that all fractions ameliorated ferrous-induced lipid peroxidation in a concentration-dependent manner. Of all the fractions of the crude MEJC, CFJC had the highest percentage inhibition of 30, 38, 49, 64 and 75% at 50, 100, 200, 400 and 800 µg/mL, respectively. The EFJC and MFJC had moderate inhibitory activities in this regard.

The effects of varying concentrations of the solvent fractions of *Jatropha curcas* on cytochrome C release is depicted in Fig. 12. As seen from the results, there was significant ($p < 0.05$) release of cytochrome C from the mitochondria following exposure to MEJC and CFJC. Again, MFJC had no effect on cytochrome C release while the effect observed at 90 µg/mL for EFJC was however not significant.

3.2 Discussion

Apoptosis is an intrinsic cell-suicide programme which ensures proper development by maintaining tissue homeostasis and safeguarding the organism by eliminating damaged or infected cells that may interfere with normal function [37]. The incidence of a number of diseases including cancer is closely connected with dysregulated apoptotic cell death. Prolific

research efforts over the last two decades, regarding elucidation of the basic mechanisms that regulate apoptosis and associated mediators that trigger or inhibit cell death have laid the foundation for therapeutic strategies of combating cancer by targeting apoptosis [38].

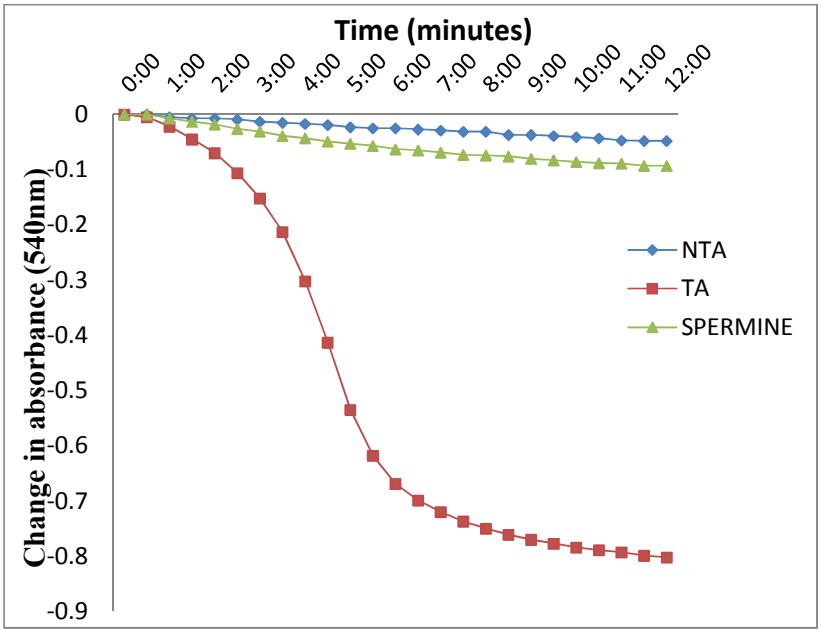

Fig. 1. Calcium-induced mitochondrial permeability transition pore opening in normal rat liver mitochondria and its reversal by spermine

*NTA: Non-Triggering Agent; TA: Triggering Agent, *Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

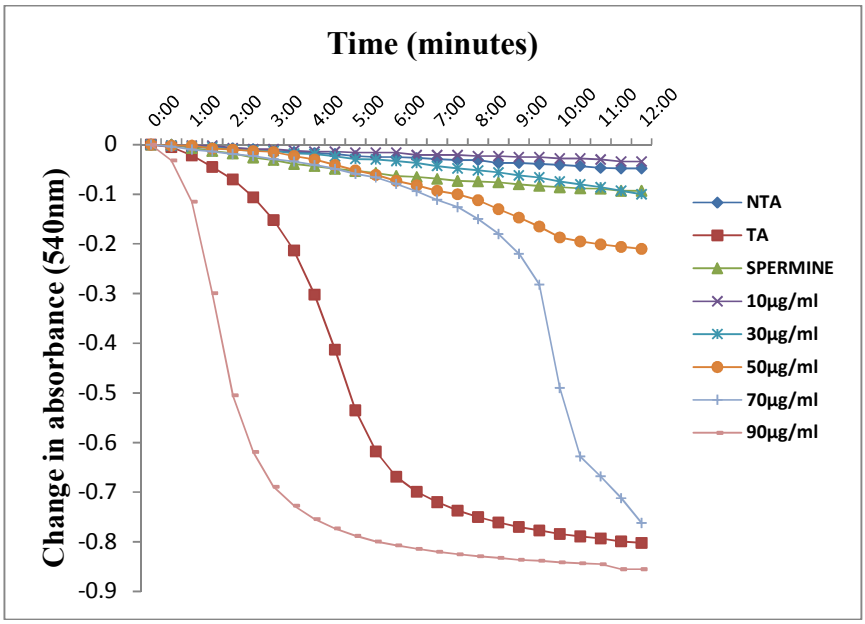

Fig. 2. Effects of varying concentrations of crude methanol extract of *Jatropha curcas* (MEJC) on rat liver mitochondrial permeability transition pore in the absence of calcium

*NTA: No triggering agent; TA: Triggering agent, *Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

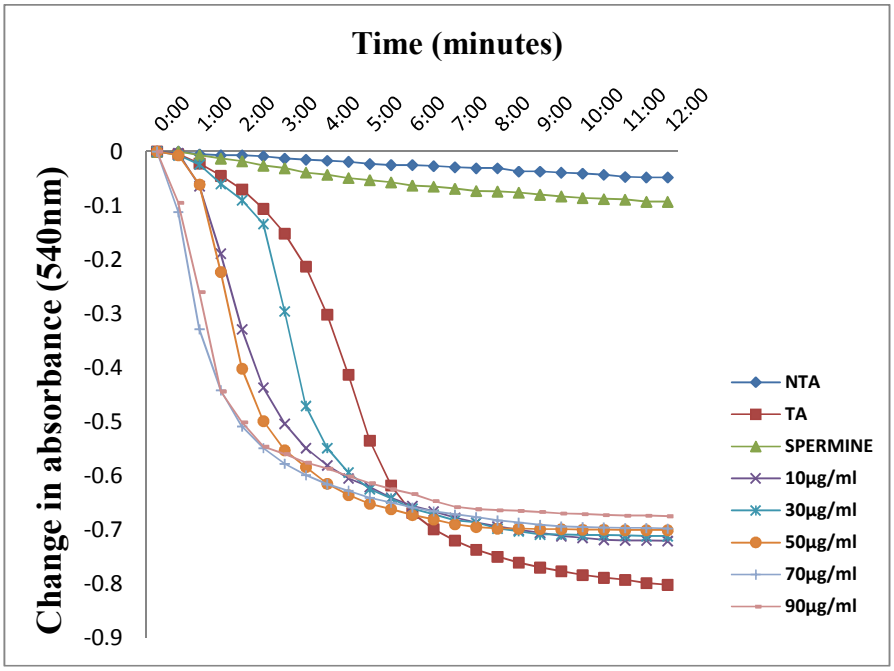

Fig. 3. Effects of varying concentrations of crude methanol extract of *Jatropha curcas* (MEJC) on the mitochondrial permeability transition pore in the presence of calcium

NTA: Non-Triggering agent; TA: Triggering agent

**Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

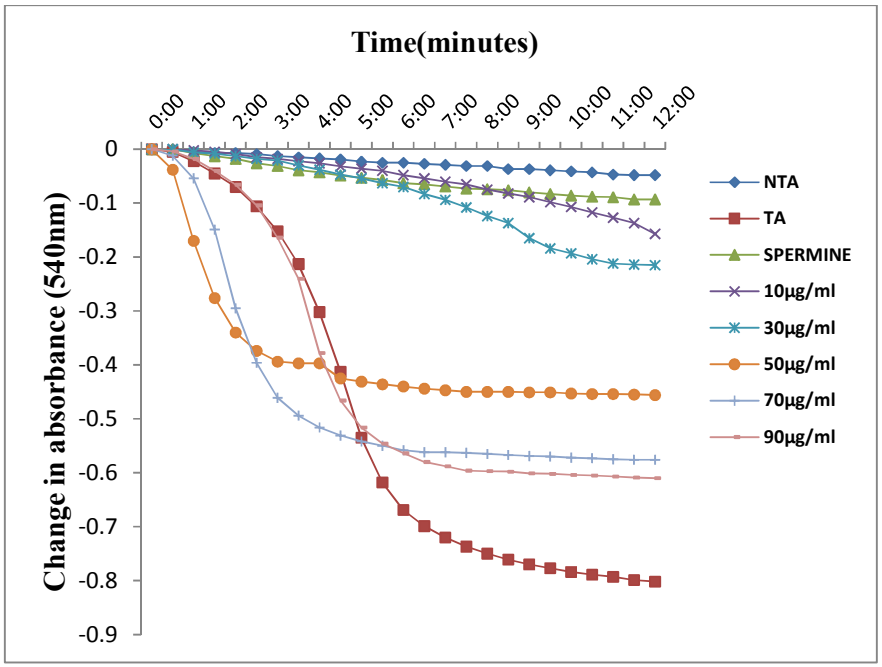

Fig. 4. Effects of varying concentrations of chloroform fraction of *Jatropha curcas* (CFJC) on the mitochondrial permeability transition pore in the absence of calcium

NTA: Non-Triggering agent; TA: Triggering agent

**Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

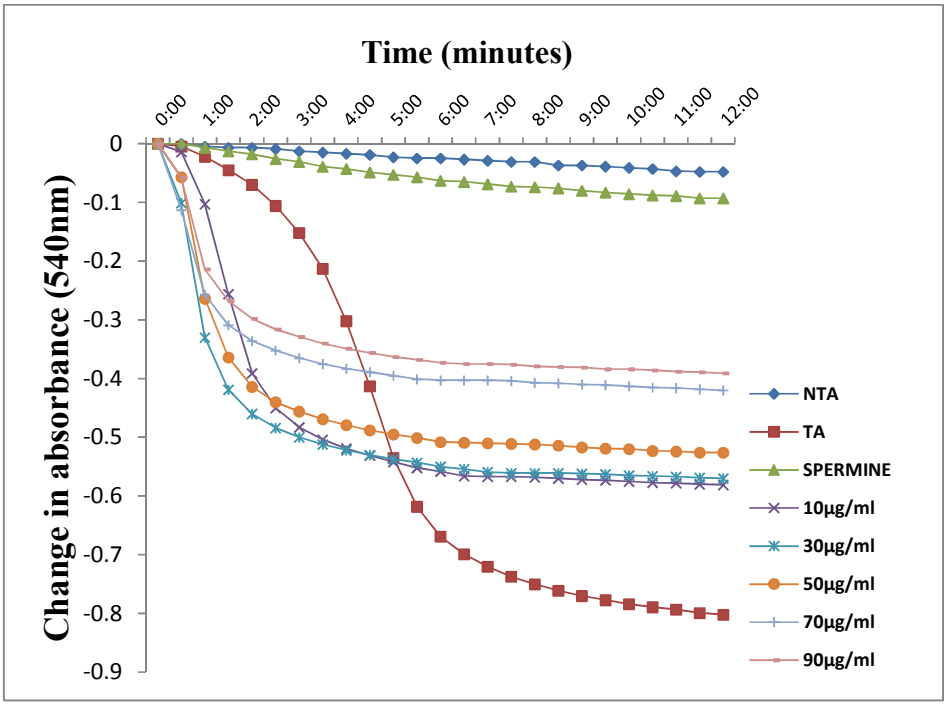

Fig. 5. Effects of varying concentrations of Chloroform Fraction of *Jatropha curcas* (CFJC) on the mitochondrial permeability transition pore in the presence of calcium

*NTA: Non -Triggering agent; TA: triggering agent, *Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

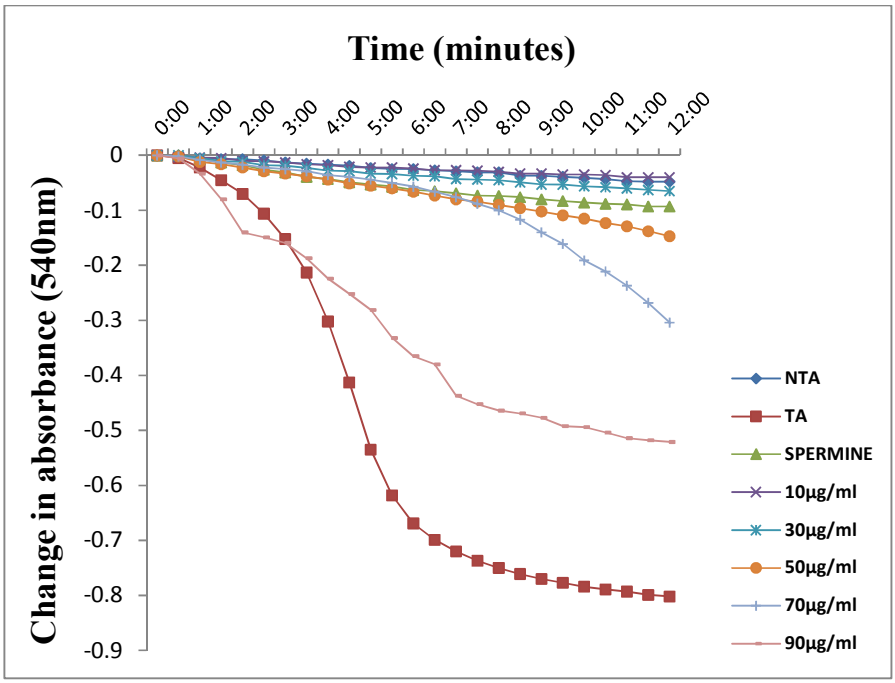

Fig. 6. Effects of varying concentrations of Ethylacetate Fraction of *Jatropha curcas* (EFJC) on the mitochondrial permeability transition pore in the absence of calcium

*NTA: Non-Triggering agent; TA: Triggering agent, *Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

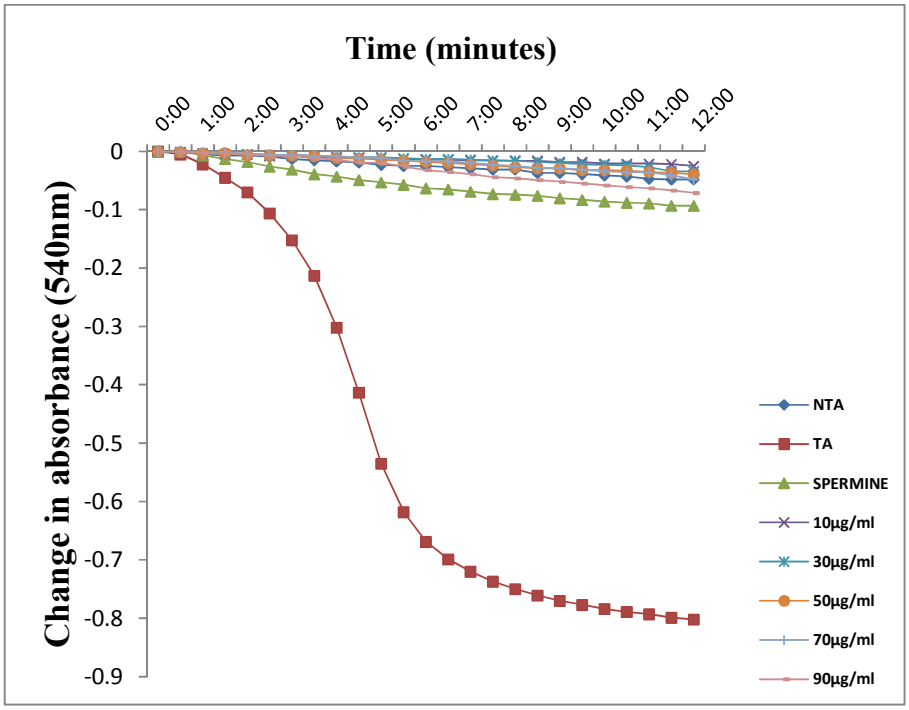

Fig. 7. Effects of varying concentrations of methanol fraction of *Jatropha curcas* (MFJC) on mitochondrial permeability transition pore in the absence of calcium

*NTA: Non-Triggering agent; TA: Triggering agent, *Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

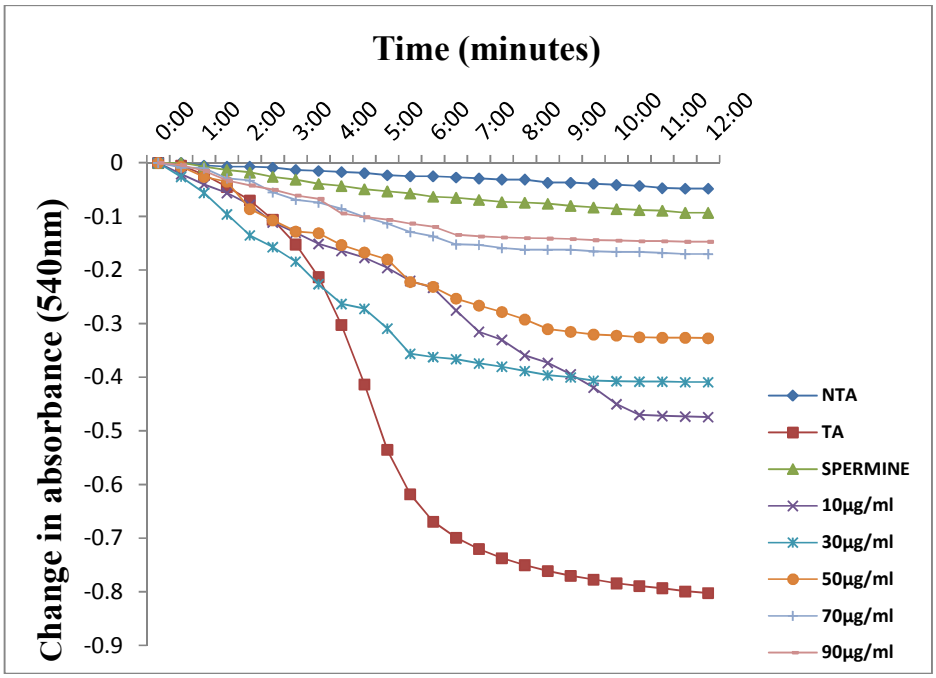

Fig. 8. Effects of varying concentrations of ethylacetate fraction of *Jatropha curcas* (EFJC) on the mitochondrial permeability transition pore in the presence of calcium

*NTA: Non-Triggering agent; TA: Triggering agent, *Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

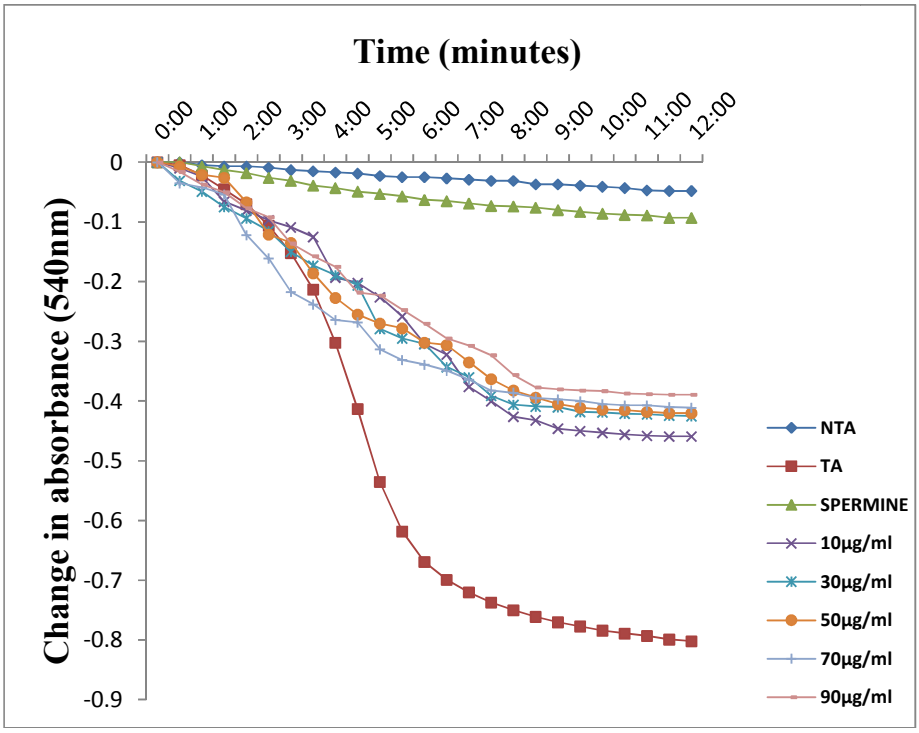

Fig. 9. Effects of varying concentrations of methanol fraction of *Jatropha curcas* (MFJC) on the mitochondrial permeability transition pore in the presence of calcium

NTA: Non-Triggering agent; TA: Triggering agent.

**Typical data of one experiment are shown and similar results were obtained in at least three different preparations.*

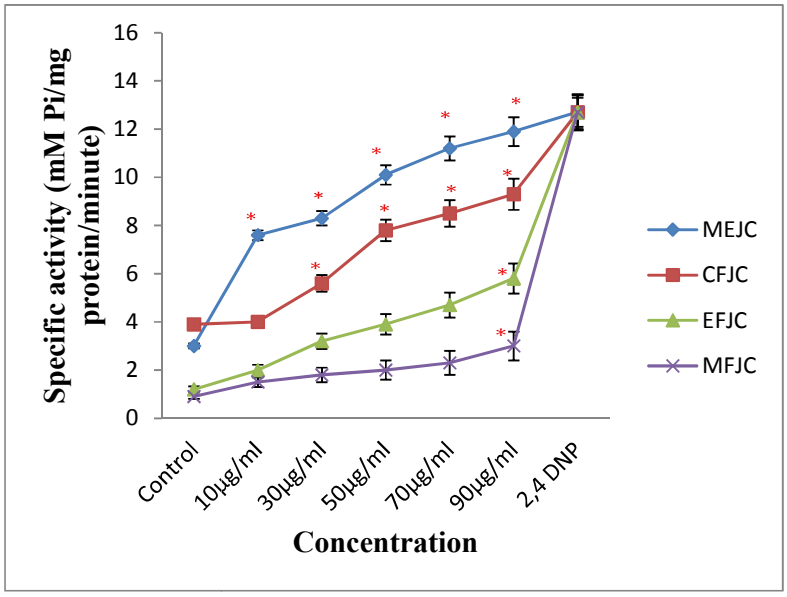

Fig. 10. Effects of different fractions of *Jatropha curcas* on specific activity of rat liver mitochondrial ATPase. 2,4DNP- 2,4-dinitrophenol.

$p < 0.05$ vs control, each value is a mean of four different determinations ± SD

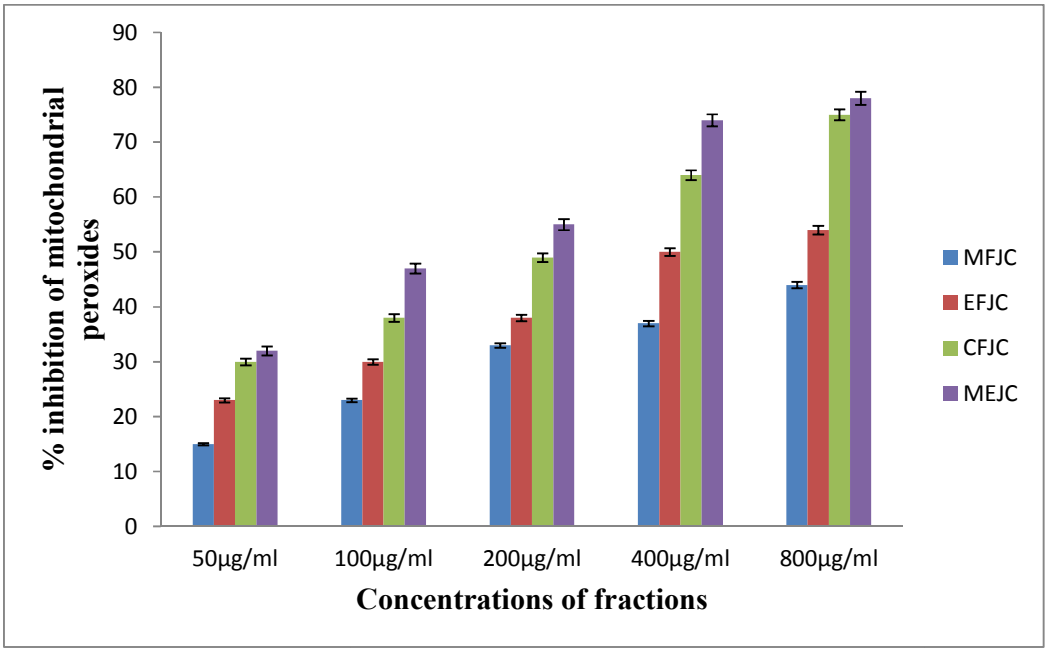

Fig. 11. Percentage inhibition of ferrous-induced mitochondrial lipid peroxidation by different fractions of *Jatropha curcas*.

Each value is a mean of 4 determinations ± SD

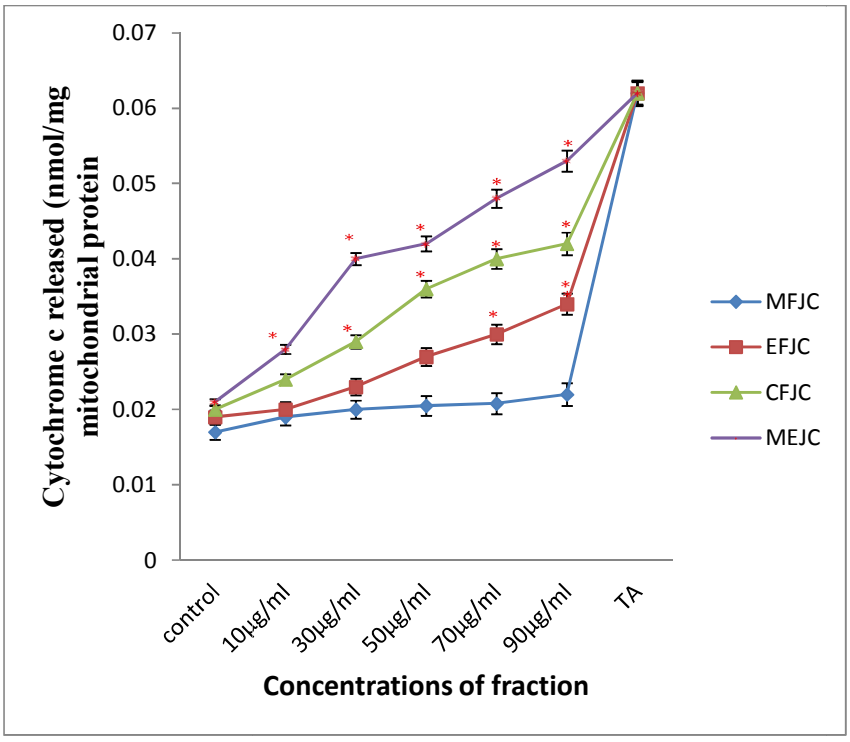

Fig. 12. Effects of solvent fractions of crude methanol extract of *Jatropha curcas* on cytochrome C release.

$p < 0.05$ vs control. Each value is a mean of four different determinations ± SD

Mitochondria have been known to play a crucial role in the induction of apoptosis because, the opening of the mitochondrial permeability transition (mPT) pore results in the release of cytochrome C and other pro-apoptotic proteins, and consequently apoptosis [9]. The mPT pore therefore serves as a useful chemotherapeutic strategy for drug development. Research indicates that certain bioactive agents present in medicinal plants e.g. epigallocatechin gallate, capsaicin in chilli pepper, quercetin in onions, resveratrol in grape, organo-sulfur compounds in garlic, and lycopene in tomatoes among many others can modulate apoptosis, especially at the stage of the permeabilization of the mitochondrial membrane [15].

In this study, the modulatory effects of the extract of *Jatropha curcas* were investigated on cytochrome C release by measuring the opening of mPT pore in isolated mitochondria. First, the susceptibility of the pore to extra mitochondrial calcium that must first enter the mitochondria was determined. This was done by monitoring the calcium-induced decrease in light scattering that reflects the mitochondrial swelling accompanying calcium-induced opening of the pore and its reversal by spermine, a standard inhibitor of pore opening [30]. Our results showed that the mitochondria used in this study were intact *ab initio* and suitable for further use. The findings that MEJC and CFJC in the absence of calcium induced significant opening of the pore in a concentration-dependent manner compared to control indicated that they may contain certain bioactive agents that could cause the assemblage of the components of the pore and thereby causing the opening of the pore. Furthermore, the observation that the opening of the pore was reversed by spermine suggests that induction of pore opening by MEJC and CFJC had no deleterious effect on membrane integrity.

The findings that EFJC and MFJC had no significant effect on mPT pore at lower concentrations suggests the presence of low concentrations of the active principle in these fractions compared to crude MEJC and CFJC. Conversely, in the presence of calcium, varying concentrations of MEJC, CFJC, EFJC and MFJC caused reversal of calcium-induced pore opening relative to spermine. This may be due to the presence of certain phytochemicals which have calcium-chelating properties, thereby reducing the concentration of calcium available to induce mPT pore opening.

The mitochondrial F_0F_1 ATPase is known to harness the proton gradient generated during the transfer of electron along the respiratory chain and couple it to oxidative phosphorylation of ADP and inorganic phosphate to produce ATP required for diverse biochemical and cellular functions [39]. The collapse in mitochondrial gradient could result in the hydrolysis of ATP by F_0F_1-ATPase for the proton gradient recovery. This turns the F_0F_1-ATPase into a consumer rather than a producer of ATP in failing cells [39]. Opening of the mPT pore has been shown to cause the release of mitochondrial factors, the dissipation of membrane potential, loss of the biochemical homeostasis of the cell and ATP hydrolysis. In order to determine the effect on pore opening by fractions of *J. curcas* on the energy status of the cell, the modulatory effect of the various fractions on mitochondrial F_0F_1 ATPase activity was investigated. The findings that ATPase activity was enhanced significantly by MEJC and CFJC agrees with our previous data on their induction of pore opening. However, the stimulatory effect displayed by EFJC was not significant while MFJC had no effect whatsoever on mitochondrial ATPase activity. These observations show that certain phytochemicals present in these fractions were able to interact with the pore and induce its opening leading to a decline in the bioenergetic status of the cell.

The release of cytochrome C from the intermembrane space as a result of the opening of the pore has been shown to be a point of no return for apoptosis [9]. In the presence of CFJC, the concentration of cytochrome C released from the mitochondria was highest at all the concentrations tested when compared to other fractions. This shows that mPT inductive effect of CFJC provoked the release of cytochrome C, apro apoptotic factor into the reaction medium. These results confirm that the opening of the pore resulted in the translocation of cytochrome C from the inter membrane space to the cytosol. These results are in consonance to our previous data on mPT that of all the fractions of the crude MEJC, CFJC had the highest inductive effect.

Compounds that inhibit membrane phospholipids peroxidation may be exerting a pharmacological effect in the prevention of radical-induced oxidative pathological events [40]. Given that reactive oxygen species have been shown to mediate mPT pore opening, we inquired if the mechanism of induction of pore opening is via generation of mitochondrial lipid peroxides. The findings that extracts of *J. curcas* inhibited the

generation of mitochondrial lipid peroxidation in a concentration-dependent manner to varying degrees rules out peroxidation of mitochondrial membranes as a probable mechanism by which these fractions elicit their inductive effect. This shows that the fractions could play a role in protecting the physiochemical properties of membrane bilayers from free radical-induced severe cellular dysfunction, possibly because they contain chemicals that act as scavengers of free radicals, like phenolic compounds, which has been shown to be correlated to the antioxidant activity of natural plant product [40]. Besides, these observations further strengthen the notion that extracts of *J. curcas* interact with the membrane in a manner that preserves its integrity while induction of mPT pore occurred.

4. CONCLUSION

Although the nature of the bioactive agent responsible for the inductive effects shown by CFJC on the mPT pore is still unknown, further work to elucidate and characterize the structure of putative agent(s) present in CFJC and their effect on induction of mitochondrial-mediated apoptosis is required. *Jatropha curcas* could regulate important mitochondrial functions and therefore be important in the management of diseases where apoptosis is downregulated.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the University of Ibadan Animal Care & Use Research Ethics Committee (ACUREC).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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