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Comparative Study of the Effects of Different Fractions of *Ficus Exasperata Vahl* on Rat Hepatic Mitochondrial Membrane Permeability Transition Pore

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ABSTRACT

A number of phytochemicals have been established as potent inducers of the mitochondrial membrane permeability transition (MMPT) pore, a transient structure formed in the inner mitochondrial membrane (IMM). There is however no information on the effect of Ficus exasperata (F. exasperata) a popular medicinal plant, on this pore, necessitating this investigation. The in vitro effects of varying concentrations (200, 400, 600, 800 and 1000µg/ml) of the methanol, n-hexane and ethylacetate fractions of leaf and stem of F. exasperata on MMPT pore opening in experimental rat liver were assessed. Opening of MMPT pore was spectrophotometrically assayed under succinate-energized condition using a modified method of Lapidus and Sokolove, 1993[34]. The results obtained for the Stem Methanol Fraction (SMF) and Leaf Methanol Fraction (LMF) show significant (P< 0.05) increases in the extents to which MMPT pore opening was induced at all concentrations compared with the control group, with the highest induction observed at 400µg/ml and the least seen at 1000µg/ml. The inductive effects of Leaf n-hexane Fraction (LHF) and Stem n-hexane Fraction (SHF) increased as concentration increased such that the highest induction observed in LHF and SHF were 2.80 and 2.28 fold increases respectively. Leaf Ethylacetate Fraction (LEF) and Stem Ethylacetate Fraction (SEF) of F. exasperata also show a concentration dependent effect such that the inductive effect decreased as concentration increased i.e. at 200µg/ml of LEF and SEF, 3.97 and 4.25-fold increases were achieved respectively while the least folds in induction were observed at 1000µg/ml for both at 2.0-fold and 1.97-fold respectively. Taken together, all tested fractions of F. exasperata have significant P < 0.05 inductive effect on the MMPT pore opening at all tested concentrations, the most potent fraction being the SEF. We thus conclude that F. exasperata is an effective inducer of the MMPT pore, albeit, different fractions derived from different parts vary in their potency. Also, F. exasperata could find a great use in the treatment of ailments dependent on the induction of the mitochondrial membrane permeability transition pore opening.

Key Words: *MMPT Pore, Ficus exasperata, Methanol, N-Hexane and Ethylacetate*

INTRODUCTION

Mitochondria are essential for human existence, and thus are involved in numerous cell processes that rely on energy sustenance such as cell growth, cell messaging, aging and replication, for this same reason, mitochondria are known to be associated with several diseases of energy demanding organs and tissues of the body including the heart, brain and skeletal muscle [1]. Thus, mitochondrial membrane potential (or proton motive force) is the central bio-energetic parameter that controls respiratory rate, ATP synthesis and the generation of reactive oxygen species, and its self-controlled by electron transport and proton leaks [2]**.** Opening of the mitochondrial membrane permeability transition (MMPT) pore causes the cell to show the characteristics of apoptosis by promoting the release of apoptotic proteins [3].

The term "mitochondrial permeability transition" refers to a sudden and irreversible increase in the permeability of the inner mitochondrial membrane (IMM) to small solutes, leading to mitochondrial trans-membrane potential ($\Delta\Psi$m) dissipation, ATP synthesis arrest, unregulated entry of water into the mitochondrial matrix, and osmotic breakdown of the organelle [4].

The MMPT Pore may function as a self-amplifying "switch" that, once activated, irreversibly commits the cell to apoptosis[5,6,7]. If cytosolic Ca^{2+} concentrations become too high, as following excessive neuronal firing or severe energy shortage, the subsequent mitochondrial Ca^{2+} overload will initiate the opening of a nonspecific pore in the inner membrane [8]. This Ca^{2+} dependent formation of the MMPT Pore represents an abrupt increase of permeability to solutes normally impermeable to the inner membrane (Mw < 1500 Da), causing osmotic swelling and rupture of the outer membrane, with concomitant loss of mitochondrial proteins [9].

Opening of the mitochondrial membrane permeability transition (MMPT) pore causes the cell to show the characteristics of apoptosis by promoting the release of apoptotic proteins[10]. Apoptosis occurs primarily via two well-recognized pathways in cells[11,12]; the extrinsic, or death receptor-mediated and the intrinsic, or mitochondrial-mediated, pathways[13]. The intrinsic apoptotic pathway depends notably on the permeabilization of mitochondrial membranes, with associated release of apoptogenic mitochondrial proteins, resulting in activation of caspase-9 and downstream cleavage of caspases 3, 6, or 7 [14] and is characterized by alteration in mitochondrial polarization and release of such mitochondrial proteins as cytochrome c, which in turn can trigger caspase activation and ultimately execution of apoptosis[15].

Ficus exasperata is a terrestrial plant that grows 20m high with smooth grey bark and very rough leaves on both surfaces; viscid non-milky sap; and coppice shoots with lobed leaves and inhabits the evergreen and optional rainforest of West Africa. It belongs to the family Moraceae with over 800 species occurring in the warmer parts of the world [16]. *F. exasperata* is commonly known as sand paper tree ("Ewe ipin" in Yoruba) and is widely spread in West Africa [17]. The plant is well known in Africa for its utilization in the treatment of different illnesses.

The leaves of *Ficus exasperata* are distichous, alternate, ovate to elliptic, subcoriaceous to coriaceous, apex shortly acuminate, base acute to obtuse, upper surface scabrous having a harsh surface, making them look like sand paper and therefore the name, sandpaper tree. The plant bears figs, which usually appear in pairs in the leaf axils. Figs are discovered either as lone or in sets in the leaf axils and rarely on matured wood. The bark of the plant is smooth grayish cream with brown streets and it radiates sticky sap. [18,19,20]

Available reports indicate that leaves of *F. exasperata* exhibit antiulcer, hypotensive, hypoglycemic, hypolipidemic, anti-inflammatory, anxiolytic, oxytocin inhibiting, anticonvulsant, antinociceptive, antipyretic, anti-microbial, anti candidal, insecticidal and pesticidal activities[21,22,23,24,2,26,27,28]. The leaves, stem bark and roots are reported to contain certain phytochemicals such as steroids, flavonoids, phlobatannins, tannins and saponins[29,30] alkaloids, cardiac glycosides,[31] and a new unnamed acylglucosylsterol[32]; unusual fatty acid from the leaves. Reports on the modulatory effects of this all important medicinal plant, *F. exasperata* on the MMPT pore opening are however nonexistent, birthing our curiosity in this present study wherein we elucidated the modulatory effect of *F. exasperata* on rat hepatic mitochondrial membrane permeability transition pore.

MATERIALS

Plant

Fresh leaves and stem of *Ficus exasperata valh* were obtained from the premises of the College of Medicine, LAUTECH Ogbomoso, Oyo state. The collected leaves were identified and authenticated by Prof. A.T.J. Ogunkunle, an Angiosperm Determinist of the Department of Pure and Applied Biology, Botany unit, Faculty of Pure and Applied Science, Ladoke Akintola University of Technology, Ogbomoso. The plant herbarium number is LHO 511.

Preparation of *Ficus exasperata* Fractions

The leaves and stems of *Ficus exasperata* were separated, rinsed with clean water, and air-dried separately in a well-ventilated room for about two weeks. Two hundred and fifty grams (250g) of leaves and about one hundred grams (100g) of stem were each pulverized using a manual blender and subsequently soaked in 1000ml each of the selected solvents to obtain the Leaf methanol fraction (LMF), Stem methanol fractions (SMF), Leaf n-hexane fraction (LHF), Stem n-hexane fraction (SHF), Leaf ethyl acetate Fraction (LEF) and Stem ethylacetate fraction (SEF). After 24hours of soaking at room temperature with occasional agitation, the macerates were filtered and concentrated to dryness in a Rotary evaporator at 65ºC and the different fractions were preserved at low temperature (4ºC) for experimental use. The yield was about 1.83% (SEF), 2.772% (LMF) and 2.678% (SMF), 0.96% (SHF), 1.83% (LHF) and 2.72% yield of (LEF) of the raw material.

Experimental Animals

Twenty healthy Wistar strain albino male rats (between 120g-150g) were purchased from commercial Breeders in Ogbomoso and kept at the Faculty of Basic Medical Sciences' Animal House, LAUTECH, Ogbomoso, Nigeria, under light-controlled conditions (12h−light/12h−dark cycle) and in well-ventilated plastic cages. The animals received formulated feeds and water ad libitum, were allowed to acclimatize over a period of two weeks and cared for in accordance with good laboratory animal care practice prescribed by the Faculty of Basic Medical Sciences' Animal Care and Use Committee.

Chemicals and Reagents

Sodium Carbonate (Na_2CO_3), Sodium Hydroxide (NaOH), Sodium-Potassium Tartarate ($Na-K-C_4O_6$), Hydrated Copper Sulphate ($CuSO_4.5H_2O$), Calcium Chloride ($CaCl_2$), Potassium Hydroxide (KOH), Methanol were Products of BDH Poole, UK Ltd. and Co., while Folin Ciocalteau Reagent, BSA, Mannitol, Sucrose, HEPES [4-(2-

Hydroxyethyl) piperazine-1-ethanesulfonic acid], EGTA, Spermine, Rotenone, and Sodium Succinate (hexahydrate) were Products of Sigma-Aldrich Co, USA. All chemicals were of analytical grade.

METHODS

Mitochondrial Isolation

The low ionic strength mitochondrion was isolated using a method described by Johnson and Lardy, 1967 [33]. The experimental animals were fasted overnight then sacrificed by "cervical dislocation" and quickly dissected. The liver was rapidly excised, trimmed to remove excess tissue and washed in homogenizing buffer (210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES, and 1 mM EGTA, pH 7.4) Thereafter, the liver was weighed, chopped and suspended in the same buffer to make a 10% suspension of tissue in buffer. Immediately the liver was homogenized on ice using a glass Bosch PSB 570-2 Homogenizer. The homogenate was sedimented twice in a Shuke (TGL-22) at 2500rpm for 5 minutes to remove the nuclear fractions and cellular debris, supernatant obtained was centrifuged at 13000rpm for 10 minutes and mitochondria fraction obtained was washed three times in washing buffer (210 mM Mannitol,70mM Sucrose, 5mM HEPES-KOH and 0.5% BSA, pH 7.4 at 12000rpm for 10 minutes. The mitochondrial pellets were suspended in swelling buffer (210 mM Mannitol, 70 mM Sucrose, and 5 mM HEPES-KOH, pH 7.4) and immediately dispensed in 1 ml Eppendorf tubes as the isolate which was used within 3 hours of isolation.

Mitochondrial Swelling Assays

Permeability transition is detected in isolated mitochondria by the change in the diffraction/absorption of light (measured at 540nm) that results from matrix swelling[8]. Thus, changes in the volume of isolated liver mitochondria were measured quantitatively at 540 nm in an SM32A spectrophotometer based on the procedure of Lapidus and Sokolove, 1993[34]. Mitochondria (0.4 mg of protein/ml) were pre-incubated in a 1 cm light path glass cuvette in the presence of 80 µM rotenone in swelling buffer containing 210 mM Mannitol, 70 mM Sucrose, 5 mM HEPES- KOH, pH 7.4 for about 3 min at 32°C prior to the addition of 300 µM $CaCl_2$ (triggering agent). Thirty seconds later, 5 mM sodium succinate was added and mitochondrial permeability was quantified as changes in absorbance at 540 nm. 4 mM spermine was added immediately after the addition of rotenone and just before the addition of mitochondria for the determination of spermine inhibition while the addition of 300 µM $CaCl_2$ was omitted in assays without triggering agent. For determination of the extents of opening of mitochondrial membrane permeability transition pore induced by the leaf and stem fractions of *F. exasperate*, varying concentrations (200, 400, 600, 800 and 1000µg/ml) of each fraction were immediately introduced after the addition of rotenone and just before the addition of mitochondria (pre-incubated with mitochondria). Absorbance readings were monitored continuously for 12 minutes based on the procedure of Lapidus and Sokolove, 1993[34]. The 12-minute cut-off was found to be optimal in obtaining a wide range of activities of the triggering agent and fractions on MMPT pore opening. Readings up to the 12th minute gave sufficient absorbance reading without loss of integrity of the mitochondrial samples[35]. Protein concentration was estimated according to the method of Lowry *et al.,* 1951[36] using bovine serum albumin (BSA) as the standard.

Statistical Analysis

Results were analyzed using student's t test. All data were expressed as Mean ± Standard Error of Mean. All results obtained for the extracts as well as that for the Triggering agent (Calcium) are significant at $P < 0.05$. Graph Pad Prism 5 and Microsoft Excel 2007 were used for the analyses.

RESULTS

In figure 1, results obtained showed that large amplitude swelling was triggered by Ca^{2+} (triggering agent) in normal healthy Wistar male rat liver mitochondria. This large amplitude swelling was observed when Ca^{2+} was added after a 3-minute incubation with mitochondria. A 9.82-fold increase in the extent of opening of the Ca^{2+} -treated mitochondrial membrane permeability pore, which is a significant induction at ($P<0.05$) was achieved. This result is in accordance with the finding that treatment of isolated mitochondrial with Ca^{2+} permeabilizes the inner membrane of mitochondria, releasing small solutes such as glutathione and $Ca^{2+[37]}$. The induction by calcium was reversed by an 80.42% inhibition in the presence of spermine, a standard inhibitor of the MMPT pore.
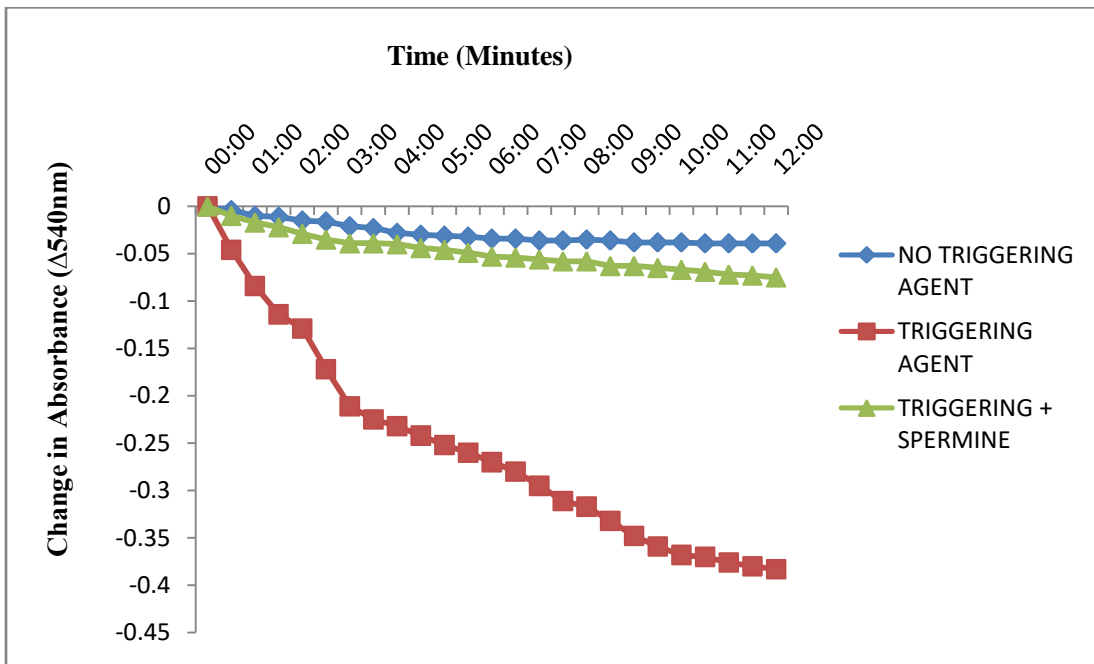
Figure 1: *In-vitro* **Induction and inhibition of Ca^{2+} -induced opening of MMPT pore in Normal Wistar strain rat liver.**

Figure 1 above shows a 9.82-fold increase in induction in the group of mitochondria treated with the standard inducer (300µM CaCl$_2$ {Trigering Agent}) when compared with the untreated control group (No Triggering Agent). This observed induction is being reversed by spermine, a standard inhibitor of the MMPT pore by 80.42%.
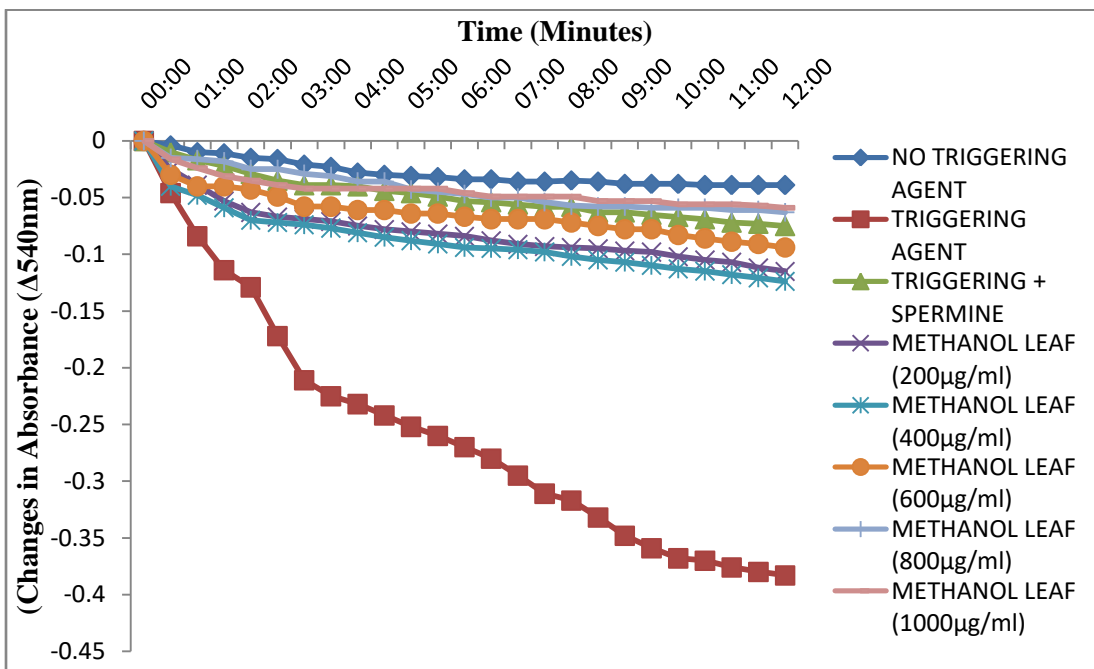

Figure 2: Showing the effect of Leaf Methanol Fraction (LMF) of *Ficus exasperata* at varied concentrations on mitochondrial Permeability Transition Pore.

Here, the in vitro induction of rat liver MMPT pore opening by varying concentrations of leaf methanol fraction (LMF) of F. exasperate are shown. Varying concentrations of LMF induced MMPT pore opening in a somewhat concentration-dependent manner, with the inductive effect decreasing with increasing concentrations of fraction such that at 200µg/ml, change in absorbance (Δ_{540nm}) was -0.115 translating to 2.94-fold increase, at 400µg/ml, Δ_{540nm} was -0.124 being a 3.17-fold increase and at 600µg/ml, $\Delta 540nm$ was -0.094 translating to a 2.41-fold increase. Furthermore, a 1.61 fold increase was observed at 800µg/ml (Δ_{540nm}= -0.063) while at 1000µg/ml, Δ_{540nm} is -0.059 translating to 1.51-fold increase. Leaf Methanol Fraction of F. exasperata causes significant ($P < 0.05$) induction at all concentration with the highest induction observed at 400µg/ml.
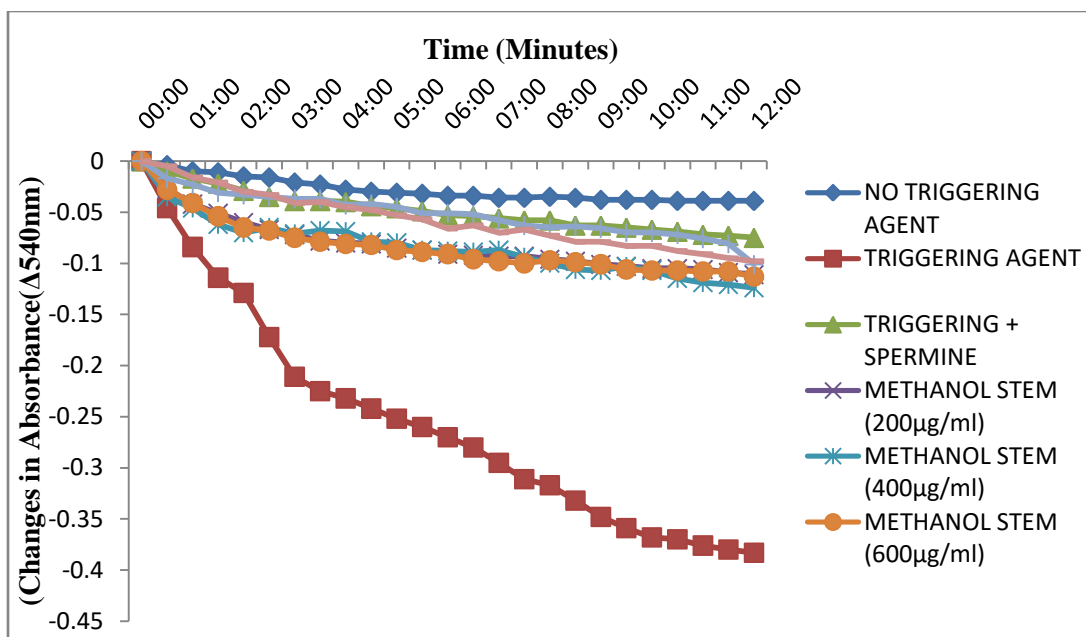

Figure 3: Showing the effect of Stem Methanol Fraction (SMF) of *Ficus exasperata* at varied concentrations on mitochondrial Permeability Transition Pore.

Figure 3 shows the in vitro induction of rat liver MMPTP opening by varying concentrations of Stem methanol fraction of Ficus exasperata. In the same vein with LMF, Varying concentrations of SMF induced MMPTP opening in a somewhat concentration-dependent manner, with the induction increasing with increasing concentrations of fraction. A 2.84-fold increase was observed at 200µg/ml (Δ_{540nm}= -0.111), at 400µg/ml, Δ_{540nm} = -0.124 (3.17-fold increase), 2.89-fold was observed at 600µg/ml with a Δ_{540nm} of -0.113, at 800µg/ml (Δ_{540nm} = -0.101) which translates to 2.58-fold increase and a 2.51-fold increase was observed at 1000µg/ml with Δ_{540nm}= -0.098. At all concentrations, Stem Methanol fractions of Ficus exasperata trigger significant ($P < 0.05$) openings of the Mitochondria Membrane Permeability Transition Pore.
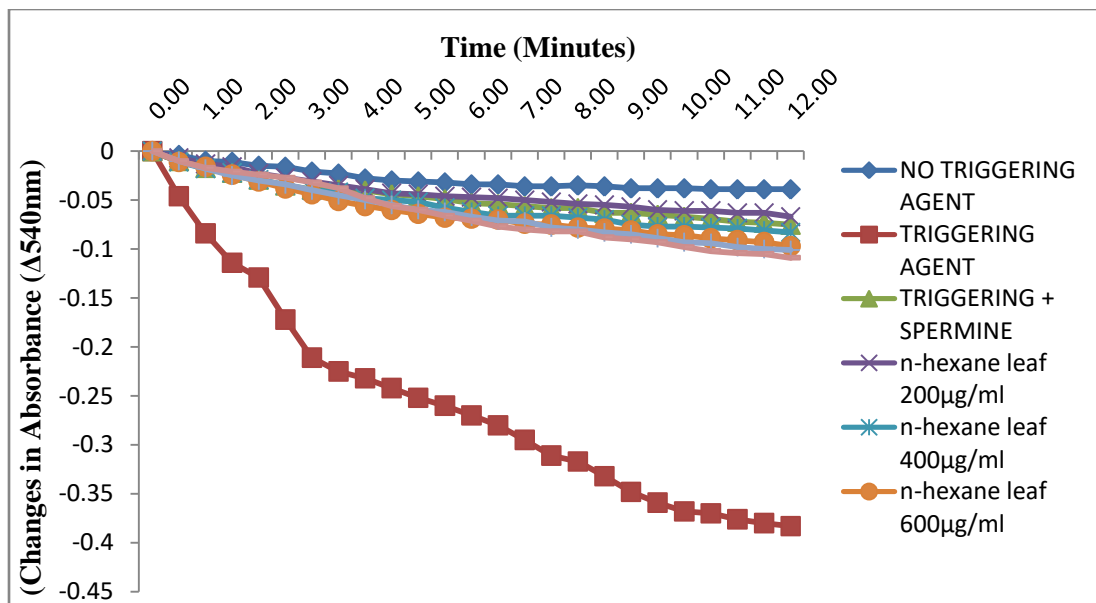
Figure 4: Showing the effect of Leaf n-Hexane Fraction (LHF) of *Ficus exasperata* at varied concentrations on mitochondrial Permeability Transition Pore.

As shown in Figure 4, all concentrations of Leaf n-Hexane Fraction of Ficus exasperata show significant (P < 0.05) induction. Such that a Δ_{540nm} of –0.067 (1.72-fold increase) was observed at 200μg/ml. At 400μg/ml, Δ_{540nm} = –0.083 (2.13-fold increase). Also at 600μg/ml, Δ_{540nm} = –0.097 (2.50-fold increase). Δ_{540nm} of -0.101 (2.59-fold increase) and Δ_{540nm} of -0.109 (2.80-fold increase) were obtained respectively at 800μg/ml and 1000μg/ml of the fraction.

Figure 5: Showing the effect of Stem n-Hexane Fraction (SHF) of *Ficus exasperata* at varied concentrations on mitochondrial Permeability Transition Pore.

According to Figure 5, The SHF has a concentration dependent inductive effect in a pattern similar to LHF i.eat 200μg/ml,Δ_{540nm} of –0.063 (1.62-fold increase); at 400μg/ml, Δ_{540nm} = –0.065 (1.67-fold increase); at 600μg/ml, Δ_{540nm} = –0.078 (2.0-fold increase); at 800μg/ml and 1000μg/ml, Δ_{540nm} of -0.080 (2.05-fold increase) and Δ_{540nm} of -

0.089 (2.28-fold increase) were obtained respectively. A significant induction of P < 0.05was observed at all concentrations.

Figure 6: Showing the effect of Leaf Ethylacetate Fraction (LEF) of *Ficus exasperata* at varied concentrations on mitochondrial Permeability Transition Pore.

As represented in Figure 6, the inductive effects of leaf ethylacetate fraction of Ficus exasperata decreases as concentration increase and a significant induction took place at all concentrations. The result observed at varying concentrations is such that at 200µg/ml, a $\Delta_{540nm} = -0.155$ (3.97-fold increase), at 400µg/ml, $\Delta_{540nm} = -0.078$ which translates to -0.135-fold increase, at 600µg/ml, 800µg/ml and 1000µg/ml, Δ_{540nm} of -0.129 (3.30-fold increase), Δ_{540nm} of -0.083 (2.12-fold increase) and Δ_{540nm} of -0.078 (2.0-fold increase) were obtained respectively.
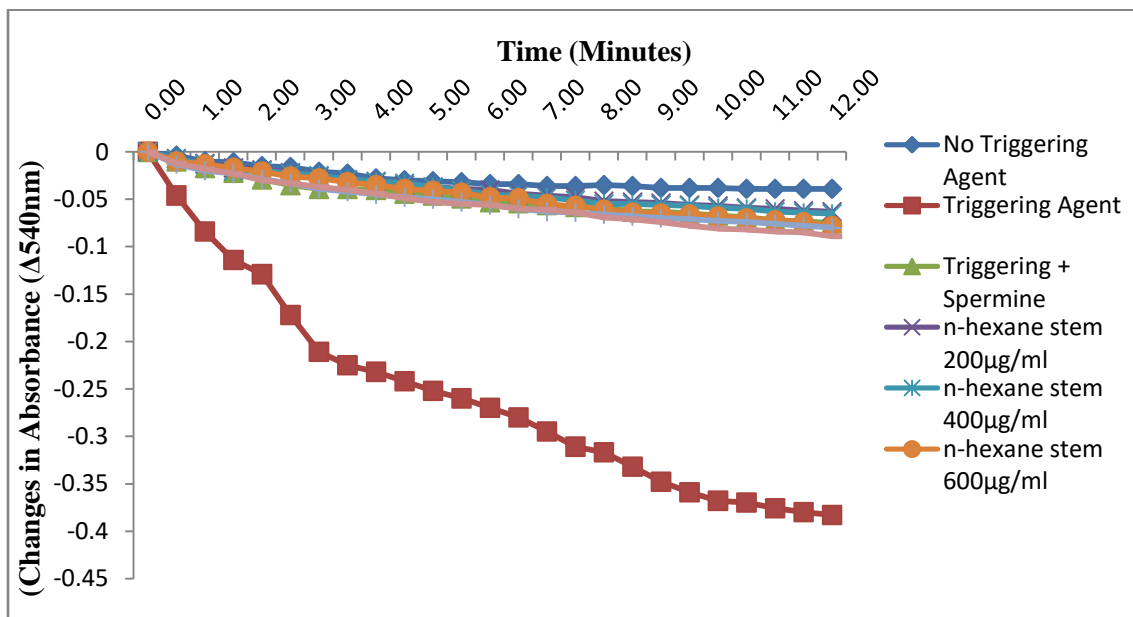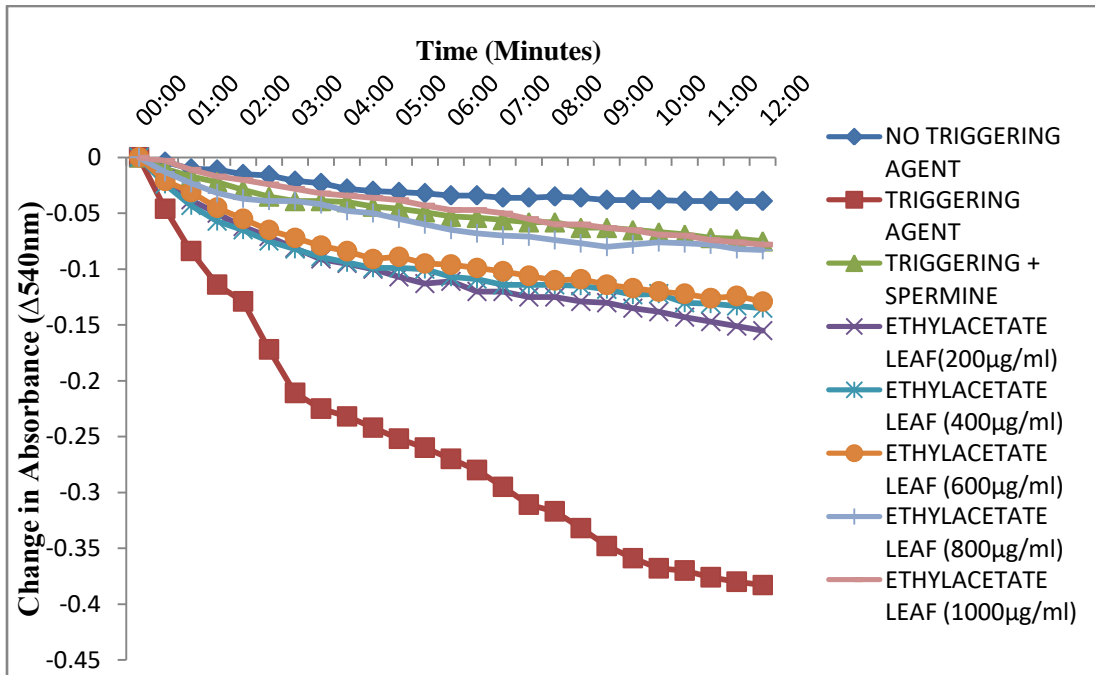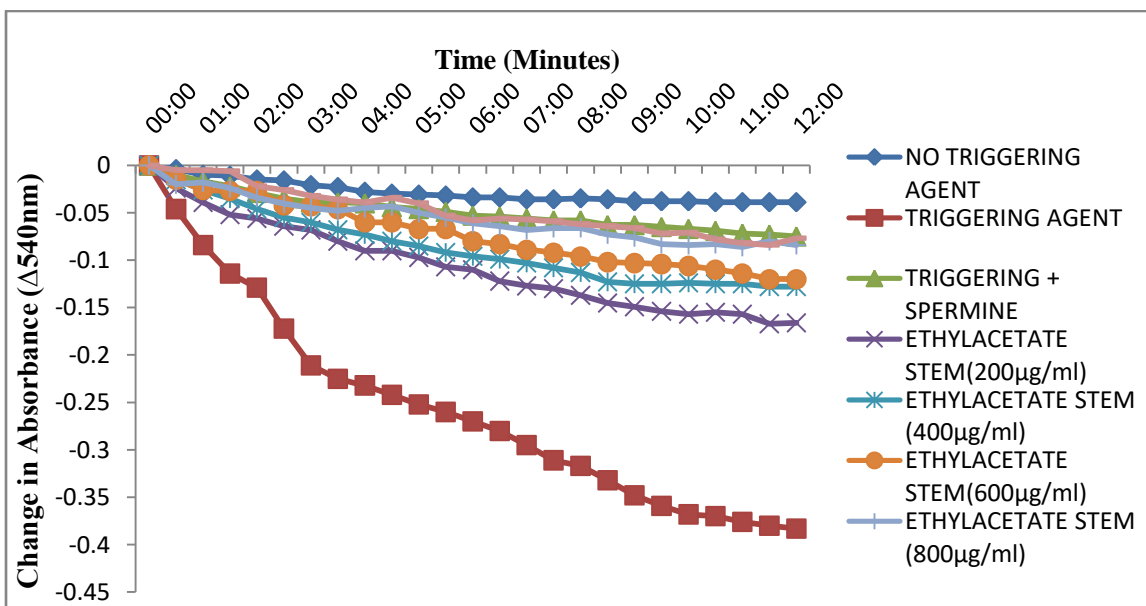

Figure 7: Showing the effect of Stem Methanol Fraction (SMF) of *Ficus exasperata* at varied concentrations on mitochondrial Permeability Transition Pore.

Figure 7 shows that the inductive effect of Stem ethylacetate fraction of Ficus exasperata decreases as concentration increase such that at 200µg/ml, 400µg/ml, 600µg/ml, 800µg/mland 1000µg/ml, Δ_{540nm}= –0.166 (4.25-fold increase), - 0.128 (3.28-fold increase), - 0.120 (3.07 fold-increase), -0.084 (2.15-fold increase), and-0.077 (1.97-fold increase) respectively.

TABLE 1: Summary of the inductive effect of Leaf Methanol Fraction (LMF) and Stem Methanol Fraction (SMF) on MMPT pore in Wistar male rat.

GROUP (n = 4)	FOLD INCREASES	CHANGES IN ABSORBANCE (Δ_{540nm})
NO TRIGGERING	1.00	-0.039±0.001472
200µg/ml LMF	2.94	-0.115±0.002041
400µg/ml LMF	3.17	-0.124±0.001633
600µg/ml LMF	2.41	-0.094±0.001414
800µg/ml LMF	1.61	-0.063±0.001225
1000µg/ml LMF	1.51	-0.059±0.001472
200µg/ml SMF	2.84	-0.111±0.004143
400µg/ml SMF	3.17	-0.124±0.00075
600 µg/ml SMF	2.89	-0.113±0.004601
800 µg/ml SMF	2.58	-0.101±0.001080
1000 µg/ml SMF	2.51	-0.098±0.001041
Triggering agent	9.82	-0.383 ±0.001080

TABLE 2: Summary of the inductive effect of Leaf n-Hexane Fraction (LHF) and Stem n-Hexane Fraction (SHF) on MMPT pore in Wistar male rat.

GROUP (n=4)	FOLD INCREASES	CHANGES IN ABSORBANCE (Δ_{540nm})
NO TRIGGERING	1.00	-0.039±0.001472
200µg/ml LHF	1.62	-0.063±0.0008332
400µg/ml LHF	1.67	-0.065±0.002056
600 µg/ml LHF	2.00	-0.078±0.001339
800 µg/ml LHF	2.05	-0.080±0.007360
1000 µg/ml LHF	2.28	-0.089±0.002483
200µg/ml SHF	1.72	-0.067±0.001225
400µg/ml SHF	2.13	-0.083±0.007070
600 µg/ml SHF	2.50	-0.097±0.001061
800 µg/ml SHF	2.59	-0.101±0.0004082
1000 µg/ml SHF	2.80	-0.109±0.001472
Triggering agent	9.82	-0.383 ± 0.001080

TABLE 3: Summary of the inductive effect of Leaf Ethylacetate Fraction (LEF) and Stem Ethylacetate Fraction (SEF) on MMPT pore in Wistar male rat.

| GROUPS (n=4)	FOLD INCREASES	CHANGES IN ABSORBANCE (Δ_{540nm})
NO TRIGGERING	1.00	-0.039 ±0.001472
200µg/ml LEF	3.97	-0.155±0.002041
400µg/ml LEF	3.46	-0.135±0.002041
600 µg/ml LEF	3.30	-0.129±0.001871
800 µg/ml LEF	2.12	-0.083±0.001080
1000 µg/ml LEF	2.00	-0.078±0.002780
200µg/ml SEF	4.25	-0.166±0.002160
400µg/ml SEF	3.28	-0.128±0.001080
600 µg/ml SEF	3.07	-0.120±0.006455
800 µg/ml SEF	2.15	-0.084±0.001633
1000 µg/ml SEF	1.97	-0.077±0.002483
Triggering agent	9.82	-0.383 ± 0.001080
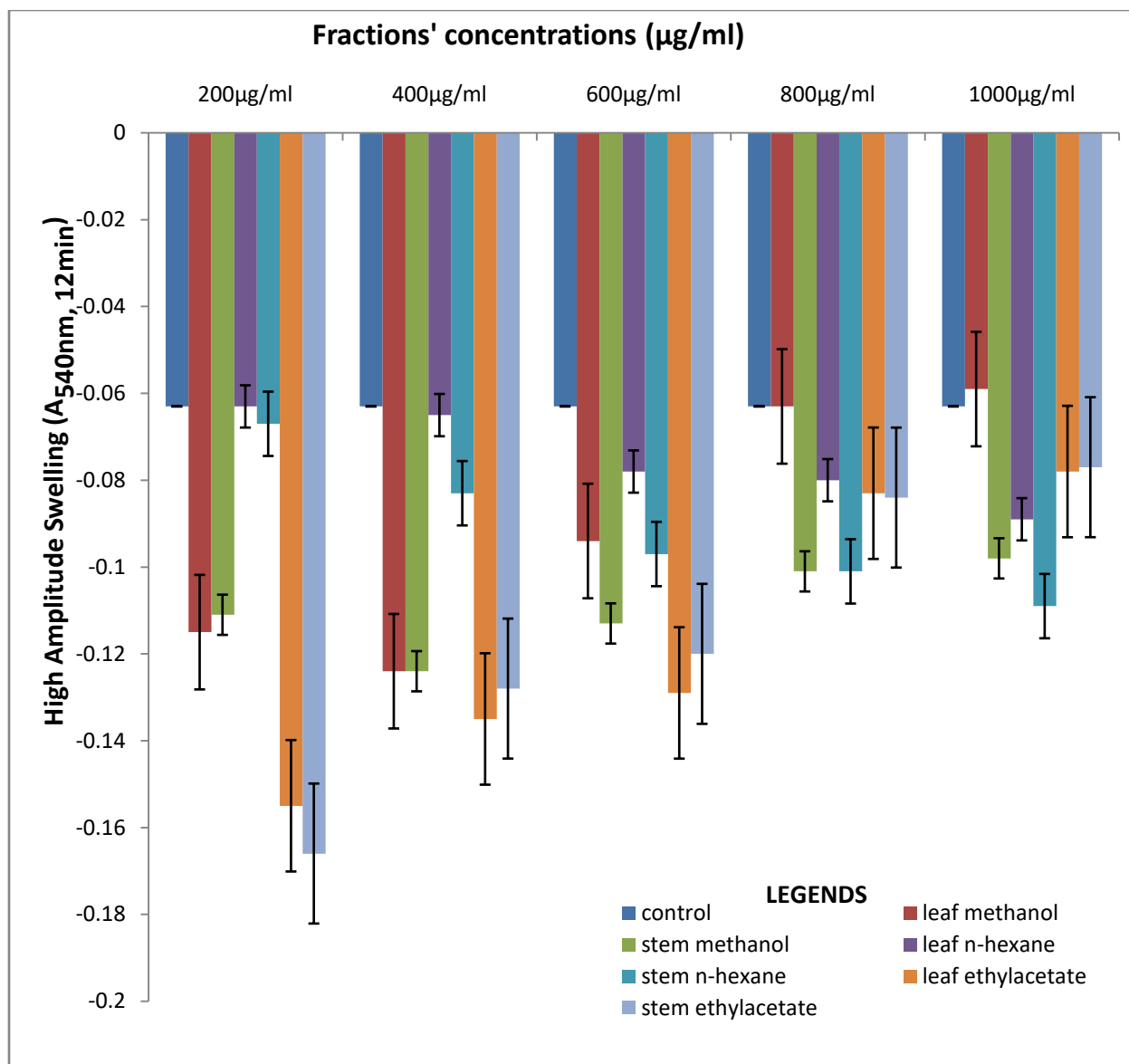
Figure 8: Comparison of the extents of induction of rat liver mitochondrial membrane permeability transition pore opening by Fractions of *F. exasperata* Bar charts shown are Means ± S.E.M of data from four separate experiments using different mitochondrial preparations.

DISCUSSION

The mitochondrion has long been identified as a critical player in both the physiology and pathophysiology of a cell. The Mitochondrial Membrane Permeability Transition (MMPT) Pore is a popular phenomenon that has been implicated in Apoptosis. Ca^{2+} is a standard triggering agent capable of stimulating the opening of this pore. Certain plants have also been identified to possess phytochemicals that can trigger the opening of the MMPT pore [38] culminating in apoptosis. Our current experiment has been able to authenticate the potency of phytochemicals in the leaf and stem of *Ficus exasperata* in the opening of the MMPT pore. *F. exasperata* induced MMPT pore opening of intact mitochondria suggesting that the presence of these phytochemicals is capable of altering the integrity of the affected mitochondrial membrane, albeit, the degrees and the patterns of induction varied for the different concentrations of the fractions that were assessed. The stem and leaf fractions of each solvent show similar pattern of induction. Among the six fractions, the SEF-treated group was the most potent as evidenced by its inductive

effect on the pore up to about 4.25-fold increase (at 200 µg/ml) in comparison with the control group. The least inductive effect was observed at 1000µg/ml of the LMF-treated group.

Arising from our gathered data; the pre-incubation of the leaf and stem of fractions of *F. exasperata* with mitochondria in the absence of Ca^{2+} induced large amplitude mitochondrial swellings, especially in the case of the SEF-treated group, we infer that the medicinal plant has bioactive agents capable of triggering apoptosis through a mitochondrial-mediated pathway. This inference is in tandem with the assertion of Chen and King, 2005[38] that the pathway of many dietary bioactive agents is via mitochondrial-mediated pathway having altered mitochondrial membrane function and/or dissipated the mitochondrial potential. Since MMPT pore may function as a self-amplifying "switch" that, once activated, irreversibly commits the cell to apoptosis, our finding suggests that the mode of induction of the pore by *F. exasperata* may not be unconnected to that known as the intrinsic apoptotic pathway in which induction of MMPT increases mitochondrial membrane permeability, causing mitochondria to become further depolarized and leading to the abolishment of membrane potential (Δψ). When Δψ is lost, protons and some molecules are able to flow across the outer mitochondrial membrane uninhibited [39,40]. Loss of Δψ interferes with the production of adenosine triphosphate (ATP), the cell's main source of energy, (because mitochondria must have an electrochemical gradient to provide the driving force for ATP production) causing osmotic swelling and rupture of the outer membrane, with concomitant loss of mitochondrial proteins [41]. Owing to the critical role played by MMPT pore opening in apoptotic cell deaths much research has found that the fate of the cell after an insult depends on the extent of MPT. If MPT occurs to only a slight extent, the cell may recover, whereas if it occurs more it may undergo apoptosis. If it occurs to an even larger degree the cell is likely to undergo necrotic cell death [42]. Though, further work is needed to ascertain the actual mode of cell death induced by our choice plant, we here conclude that *F. exasperata* is a potent inducer of the mitochondrial membrane permeability transition pore; a critical actor in a cell's life-death balance. This thus suggests a potential use for *F. exasperata* in drug developments for ailments which will benefit from MMPT pore induction.

CONCLUSION

This work suggests that the pre-incubation of fractions derived from Leaf and Stem of *Ficus exasperata* to isolated mitochondria has an inductive effect on MMPT pore opening at different concentrations (200-1000µg/ml). Although, they are less potent compared to Ca^{2+} in their ability to trigger the opening of the Mitochondria Membrane Permeability Transition Pore, the fractions clearly induced large amplitude swellings of the isolated mitochondria, an indication that the plants' phytochemicals may be of use in the management/treatment of pathological conditions such as cancer that may benefit from the killing (via apoptosis or necrosis) of the diseased cells.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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