EFFECTS OF CERTAIN FRACTIONS OF *Ocimum gratissimum* (L) LEAF EXTRACT ON RAT LIVER VIA MITOCHONDRIAL MEDIATED APOPTOSIS

(*In vitro*)

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OUTLINE

- INTRODUCTION
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INTRODUCTION

- Medicinal plants have been used in folklore medicine for centuries. This is due to metabolites which are bioactive agents such as flavonoids, polyphenols, alkaloids, saponin, terpenes among others (Blomhoff *et al.*, 2006).

MITOCHONDRIA

- Mitochondria are very unique organelles that are indispensable for maintaining energy homeostasis in eukaryotic cells, as well as play prominent roles in cell physiology and fate, including the distinction between survival and apoptotic pathways (Wu *et al*., 2010; Elekofehinti and Kade, 2012).

- Mitochondrial Permeability Transition (MPT) refers to the massive swelling and depolarization of mitochondria that occurs under some conditions, mostly resulting from calcium overload and oxidative stress (Javadov and Karmazyn, 2007).

Ocimum gratissimum (OG)

- It belongs to family Lamiaceae and commonly found in the rain forest of Nigeria or during raining season almost all parts of Nigeria.
- The fresh leaves are used as a laxative, while infusion serves as a relief for headaches, fever, diarrhea, dysentery, pile and convulsion (Danziel, 1996)
- Some of the vernacular names in Nigeria include: (Nchu-awu) Igbo, (*Efinrin*) Yoruba, and (*Daidoya*) Hausa (Effraim *et al*., 2000).
- *Ocimum gratissimum* has been reported to possess some bioactive components which include alkaloids, flavonoids, phlobatannins, steroids, anthraquinones, saponins and tannins, terpenoids (Akinmoladun *et al*., 2007).

Plate 1: Picture of *Ocimum gratissimum*

JUSTIFICATION

The justifications for this research:

- Attempts are being made on how to cure or manage various human ailments.

- The most common of these is the intervention of medical sciences which are limited due to inadequate drug supply, side effect of drugs and inability to afford such treatment especially in the developing countries (Maritim *et al*., 2003).

- It is therefore necessary to harness and develop efficacious non-toxic affordable chemotherapeutic agents from the abundance of various bioactive components of medicinal plants of which OG is a candidate (Egesie *et al*., 2016).

- OG has been used in the treatment of various disease conditions traditionally.
- However, its role on mitochondrial membrane permeability transition pore remains to be elucidated.
- Hence, the objective of the study is to evaluate whether certain fractions of OG leaf extract reverse or induce apoptosis in rat liver via mitochondrial mediated apoptosis

AIMS AND OBJECTIVES

- The objectives of this research include:
- To carry out qualitative and quantitative phytochemical screening of *Ocimum gratissium* leaf extracts.
- To determine the effects of the *Ocimum gratissimum* leaf extracts on rat liver mitochondrial membrane permeability transition (MMPT) pore.
- To quantify cytochrome c release from MMPT pore using *Ocimum gratissimum* leaf extracts.
- To determine the effects of *Ocimum gratissimum* leaf extracts on lipid peroxidation.

MATERIALS AND METHODS

PLANT MATERIAL

- **Source of plant material:** *Ocimum gratissimum* leaves were bought from Bodija Market, Ibadan and authenticated by the Department of Pharmacognosy, University of Ibadan (Voucher specimen number: DPUI No 1504).
- **Preparation of plant materials**: It was dried at room temperature (28 – 30^{o}C) for four (4) weeks (30 days) and pulverized to a fine powder using a mortal and pestle.
- The powdered leaves were stored at room temperature in a clean bottle.

Extraction:

- The extract was obtained by cold extraction method with methanol in 1:10 (w/v) ratio. After thoroughly mixed, it was allowed to stand for 72 hours and filtered using sterile whatmann No 1 filter paper.
- The green colour filtrate (extract) was concentrated using rotary evaporator. The resulting crude extract was evaporated to dryness using water bath.
- The crude methanol extract was partitioned successively between n-hexane, chloroform, ethyl acetate and distilled water.

EXPERIMENTAL ANIMALS

- Male albino rats (Wistar strain) each weighing between 100-130g were obtained from the Veterinary Medicine Animal House, Veterinary Department, University of Ibadan, Nigeria.

- The rats were kept in ventilated cage with 12 hours light/dark cycle and were given rat chow and water *ad libitum* two weeks for acclimatization before the experiment.

METHODOLOGY/ PROCEDURES

- **Isolation of rat liver mitochondria**: Low ionic strength mitochondria were isolated according to the method described by Lapidus and Sokolove (1993).
- **Protein determination:** Mitochondrial protein was estimated according to Lowry *et al.*, (1951).
- **Cytochrome C quantification:** Cytochrome c quantification was carried out essentially as described by Appaix *et al*., (2000).
- **Lipid peroxidation determination:** A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using mitochondria as lipid-rich media, as described by Ruberto *et al*., (2000).
- Phytochemical screening was carried out by simple qualitative and quantitative standard methods (Harborne, 1973; Trease and Evans, 2002).

ASSESSMENT OF THE EFFECT OF *OCIMUM GRATISSIMUM (L)* EXTRACTS ON MITOCHONDRIAL MEMBRANE PERMEABILITY TRANSITION PORE IN RAT LIVER

- Mitochondrial swelling was determined according to the method of Lapidus and Sokolove (1993).
- **PRINCIPLE:**

The principle is based on the fact that when the mitochondria swell, the increase in the volume of the mitochondrial matrix is determined spectrophotometrically by measuring the change in absorbance at 540nm.

ASSESSMENT OF CYTOCHROME C RELEASE

- Cytochrome c quantification was carried out essentially as described by Appaix *et al*., (2000).
- **PRINCIPLE**: This is based on the fact that when cytochrome c is released, the heme-containing moieties of the protein absorb light maximally at Soret peak, wavelength 414nm, which is detectable using UV-spectrophotometer.

ASSESSMENT OF THE EFFECT OF *OCIMUM GRATISSIMUM (*L*)* EXTRACTS ON Fe^{2+}-INDUCED LIPID PEROXIDATION

- Lipid peroxidation was assessed according to the method described by Ruberto *et al*., (2000).
- Principle: This is based on the complex colour formation between TBA and the MDA released from the lipid peroxide during lipid peroxidation. The absorbance is measured at 532nm

STATISTICAL ANALYSIS OF DATA

- Data were expressed as mean ± standard deviation (SD) of at least three independent measurements (assays).
- One way analysis of variance (ANOVA) and Duncan's multiple Range Test (DMRT) were carried out.
- $p<0.05$ was considered as statistically significant.

RESULTS

RESULTS AND DISCUSSIONS

EXPERIMENT 1

Qualitative and Quantitative Phytochemical Screenings of *Ocimum gratissimum* leaf

- The leaf of *Ocimum gratissimum* was screened phytochemically to ascertain the bioactive components present.

Table 1: Qualitative Phytochemical Screening of *Ocimum gratissimum* leaf.

Parameters	**Aqueous Fraction**	**Chloroform Fraction**
Alkaloids	-	+
Steroids	+	+
Flavonoids	+	+
Saponin	+	-
Resins	+	+
Phenols	+	+
Tannins	+	-

Key: -: Absent +: Present

Table 2: Quantitative Phytochemical Screening of *Ocimum gratissimum* leaf.

Parameters	**Aqueous Fraction (%)**	**Chloroform Fraction (%)**
Alkaloids	-	9.20
Steroids	0.56	0.70
Flavonoids	0.82	7.60
Saponin	0.86	-
Resins	0.88	0.76
Phenols	1.80	9.60
Tannins	7.60	-

EXPERIMENT 2

Assessment of the Effects of $Ca^{2+}$and Spermine on Rat Liver Mitochondrial Membrane Permeability Transition Pore.

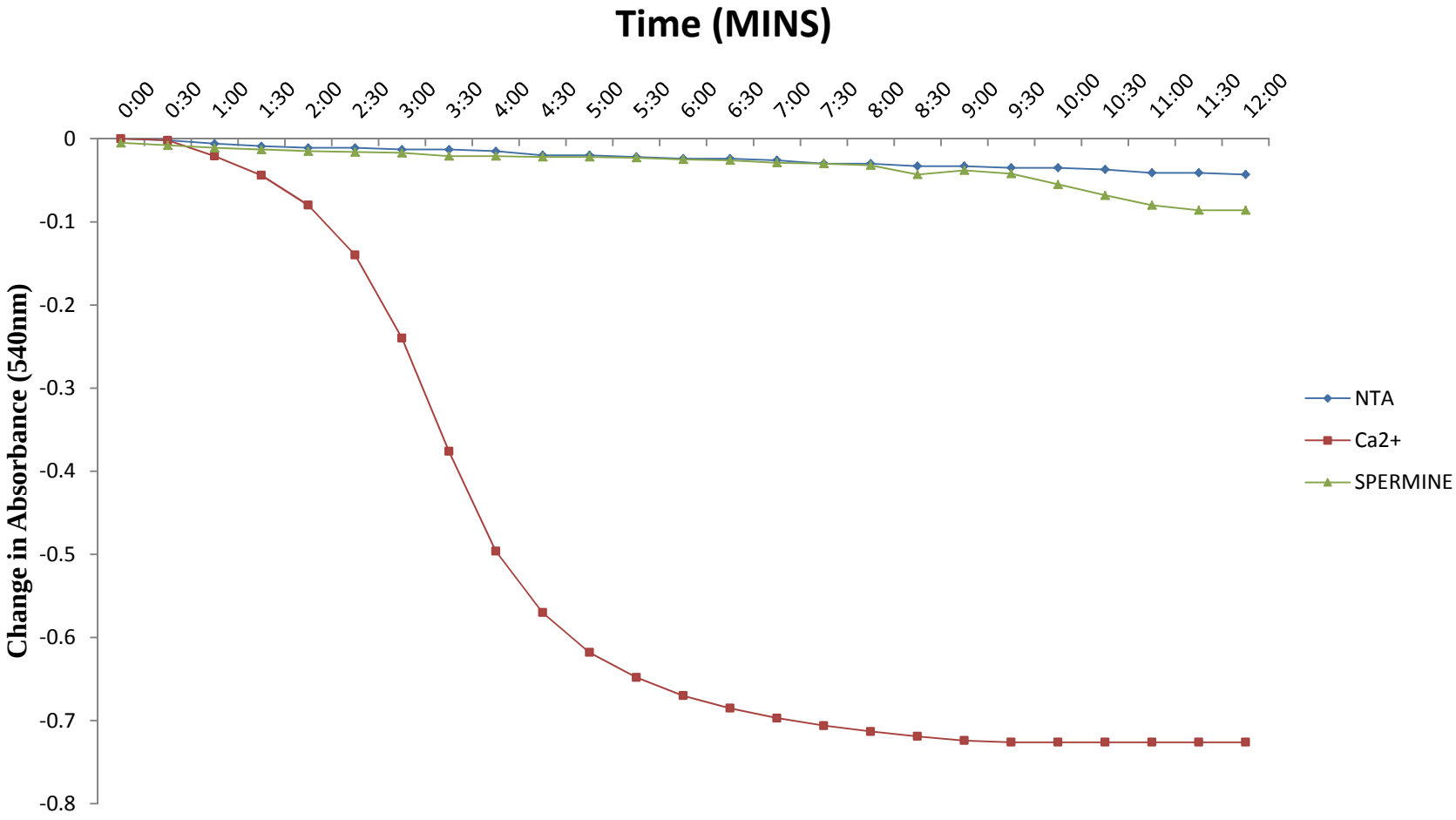

Fig. 2: Assessment of Effects of $Ca^{2+}$and Spermine on Rat Liver Mitochondrial Membrane Permeability Transition Pore.

NTA: Non -Triggering Agent

- Figure 2 shows that mitochondrial membranes were intact in the absence of Ca^{2+}, whereas presence of Ca^{2+} caused significant swelling of the membrane, thus, opening of the mitochondrial membrane permeability transition pore.
- Spermine inhibited or reversed exogenous calcium-induced mitochondrial swelling in normal rat liver.
- This reversal was significant with percentage inhibition of approximately 86.2%.

CONCLUSION

- Ca^{2+} significantly induced mitochondrial membrane permeability transition (MMPT) pore while spermine inhibited MMPT pore.
- This shows that the mitochondrial membrane integrity has not been compromised or uncoupled and therefore suitable for use.

EXPERIMENT 3

- Assessment of Effects of Methanol Extract of *Ocimum gratissimum* leaf on Mitochondrial Membrane Permeability Transition pore in the absence and presence of Ca^{2+}.

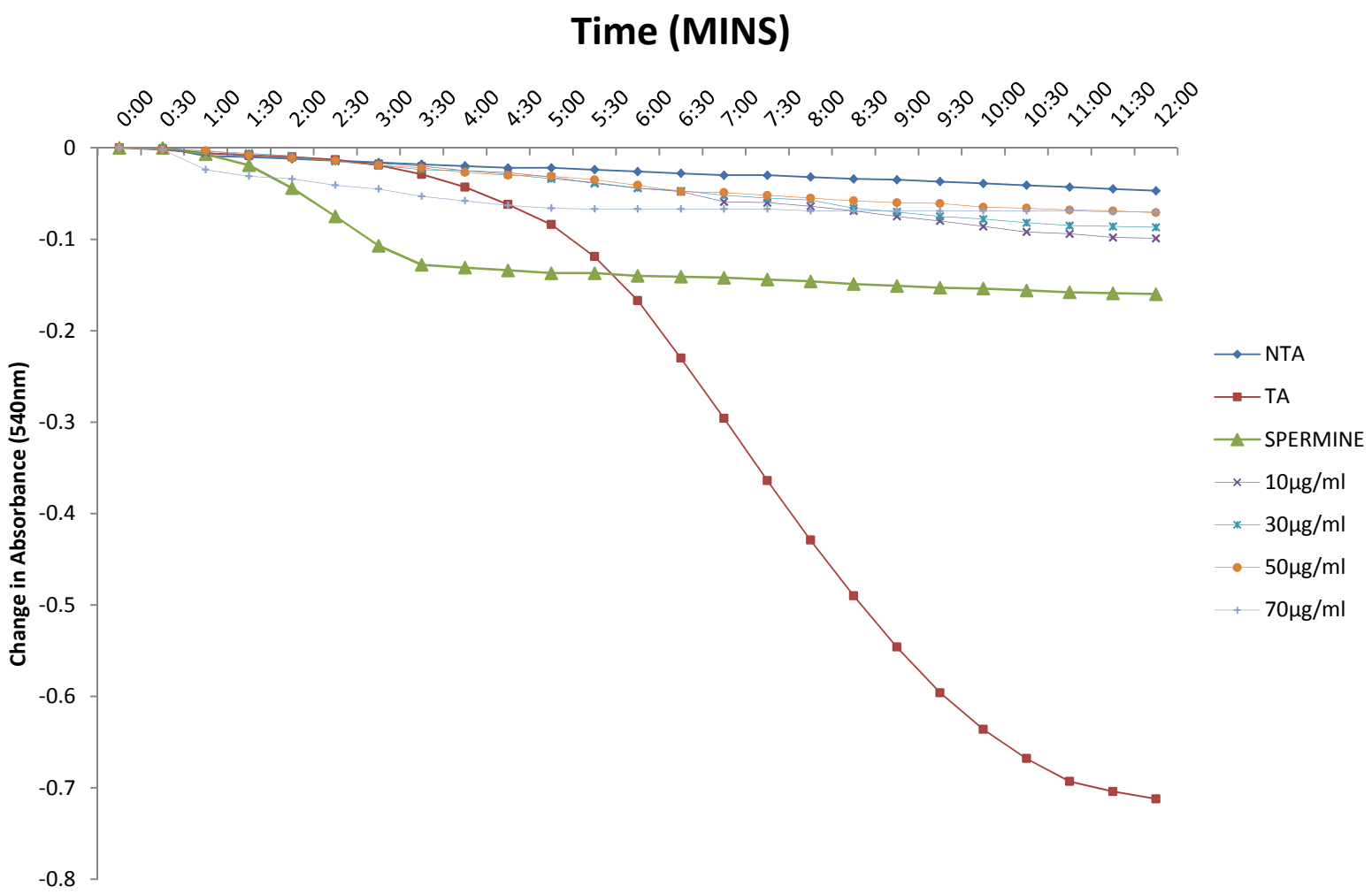

Fig. 3: Assessment of Effect of Methanol Extract of *Ocimum gratissimum* Leaf on Mitochondrial Membrane Permeability Transition pore in the absence of Ca^{2+}

Summary

- Fig. 3 shows the effect of methanol extract of *Ocimum gratissimum* leaf on mitochondrial membrane permeability transition pore in the absence of calcium.
- Mitochondria in the absence of calcium were intact. Significant pore opening was triggered by calcium whereas spermine also reversed the calcium-induced pore opening significantly.
- Treatment of the intact mitochondria with various concentrations (0.05µg/ml, 0.5µg/ml, 1µg/ml, 2µg/ml) of methanol extract of the plant showed no effect on the mitochondrial membrane as there was no significant difference from the intact mitochondria.

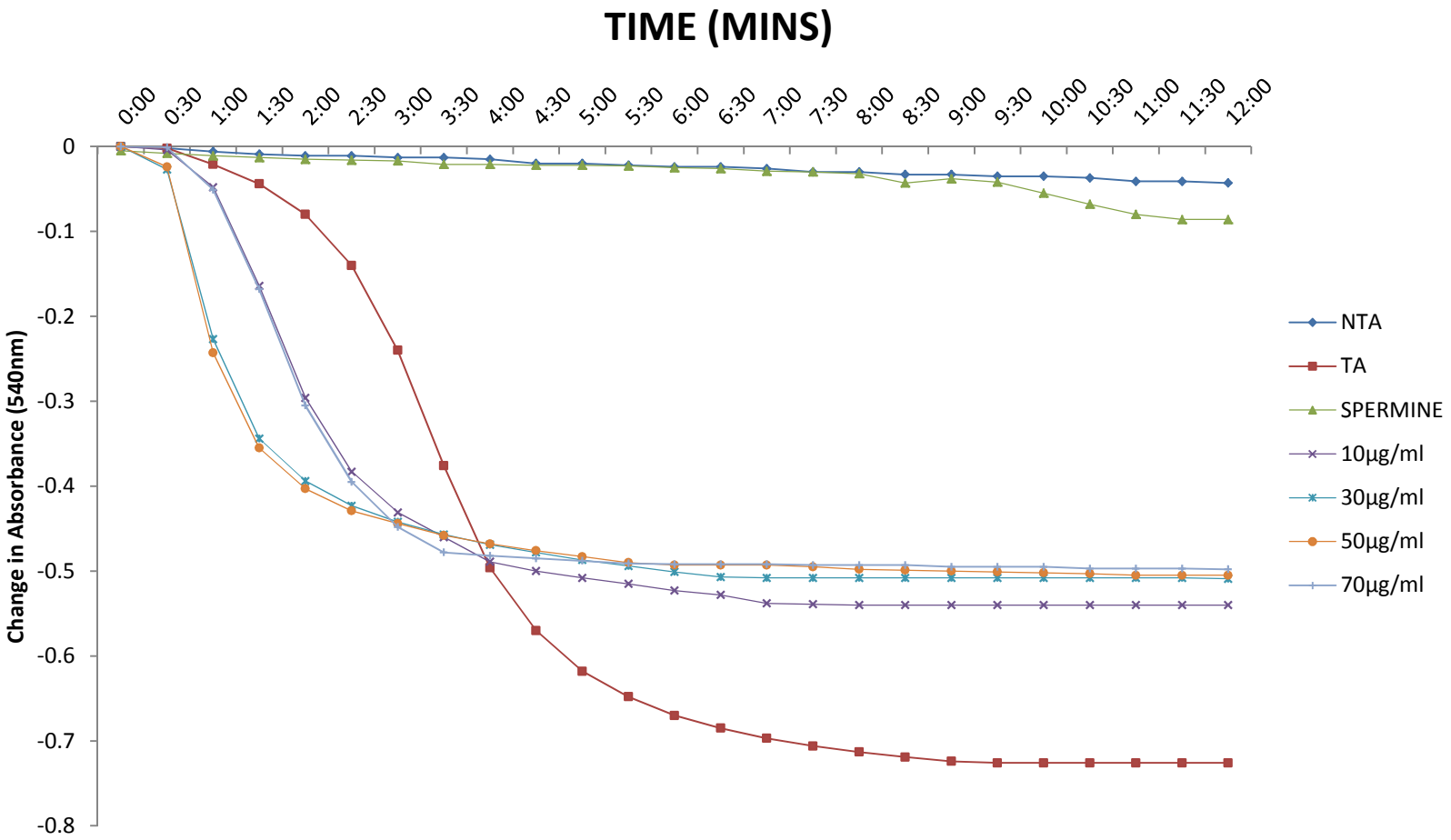

Fig. 4: Assessment of Effect of Methanol Extract of *Ocimum gratissimum* Leaf on Mitochondrial Membrane Permeability Transition pore in the presence of Ca^{2+}

Summary

- Fig. 4 shows the effect of methanol extract of *Ocimum gratissimum* leaves on calcium-induced opening of MMPT pore.
- The result shows that there was significant induction of the pore opening triggered by calcium and subsequent reversal of the opening by methanol extract of the plant
- The reversal was 25.6%, 30%, 30.4% and 31.5% by 10µg/ml, 30µg/ml, 50µg/ml and 70µg/ml respectively in concentration dependent manner.

EXPERIMENT 4

- Assessment of the Effects of Aqueous Fraction of *Ocimum gratissimum* leaf on Mitochondrial Membrane Permeability Transition pore in the absence and presence of Ca^{2+}.

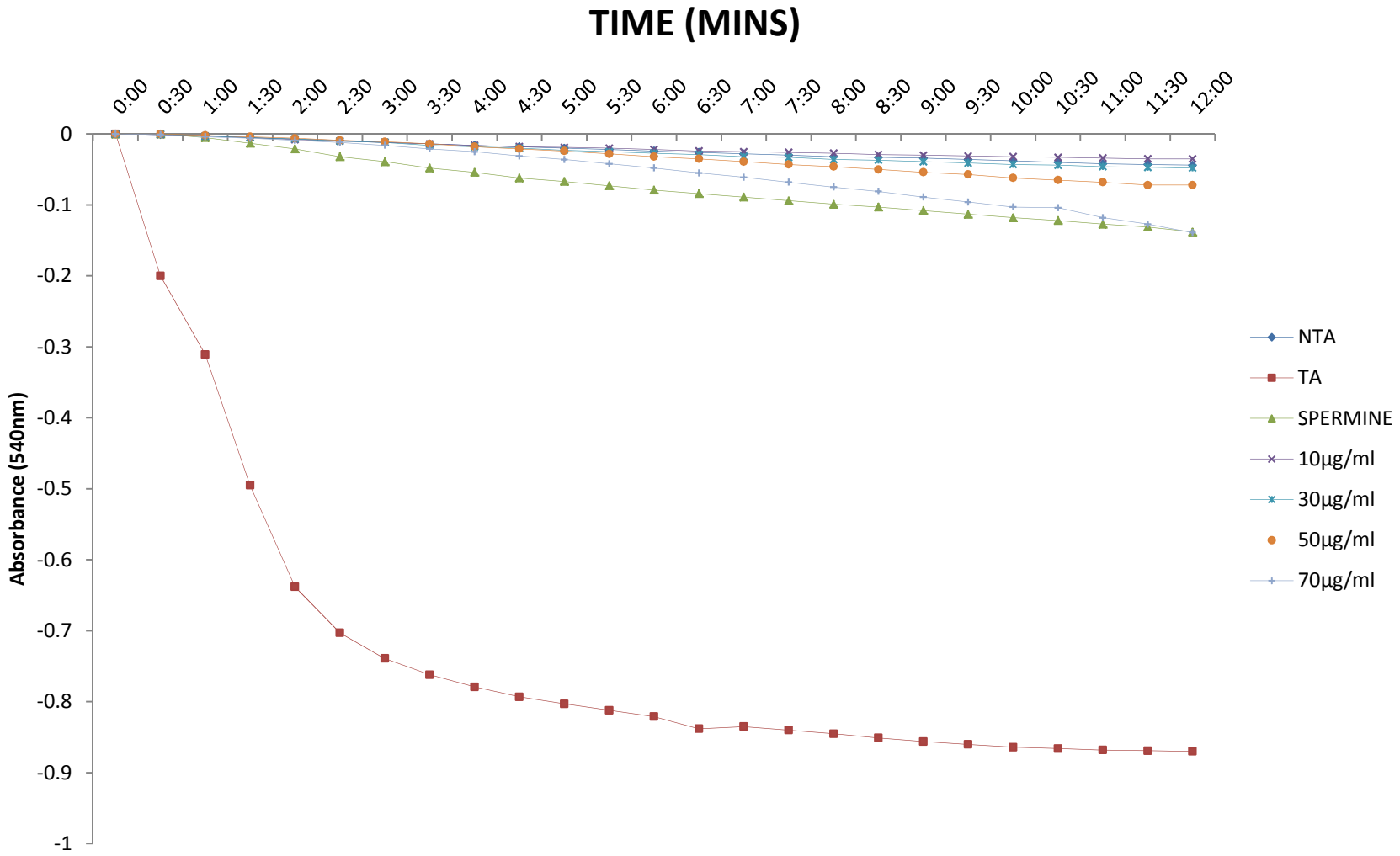

Fig. 5: Assessment of Effect of Aqueous Fraction of *Ocimum gratissimum* Leaf on Mitochondrial Membrane Permeability Transition pore in the absence of Ca^{2+}

Summary

- Fig. 5 displays the effect of aqueous fraction of *Ocimum gratissimum* leaves on mitochondrial membrane permeability transition pore in the absence of calcium.
- Treatment of the intact mitochondria with various concentrations(10µg/ml, 30µg/ml, 50µg/ml and 70µg/ml) of aqueous fraction of the plant showed no inductive effect on the mitochondrial membrane as there was no significant difference from the intact mitochondria.

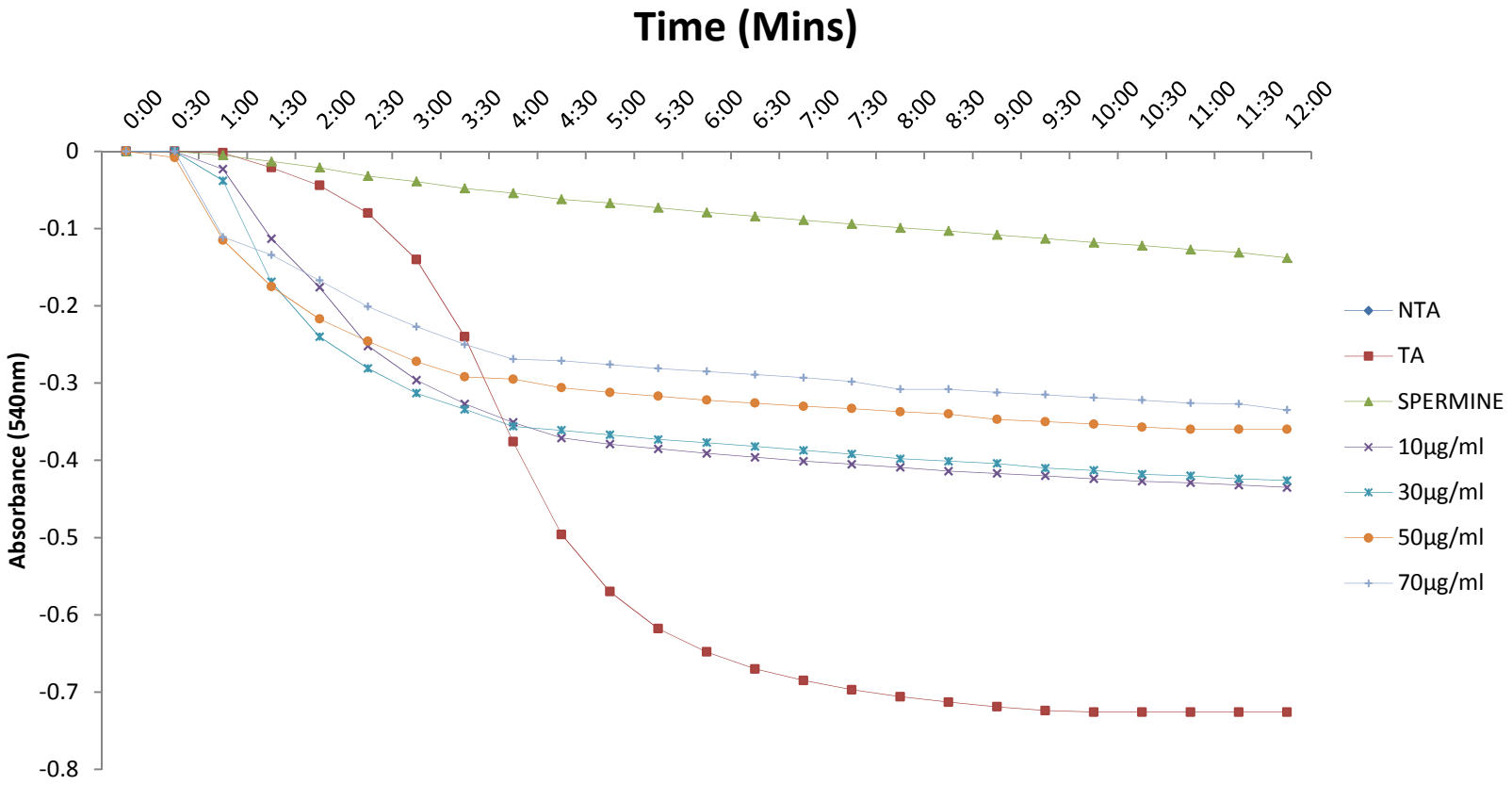

Fig. 6: Assessment of Effect of Aqueous Fraction of *Ocimum gratissimum* Leaf on Mitochondrial Membrane Permeability Transition pore in the presence of Ca^{2+}

Summary

- Fig. 6 represents the effect of the aqueous fraction of *Ocimum gratissimum* leaves on calcium-induced opening of MMPT pore.
- Mitochondria in the absence TA was intact (coupled). Treatment with Ca^{2+} significantly opened the MPT pore, while treatment with spermine was shown to significantly reverse the pore opening by Ca^{2+}.
- Treatment with 10µg/ml, 30µg/ml, 50µg/ml and 70µg/ml of the plant aqueous fraction produced inhibition of 50, 51, 58.6 and 61.5% respectively depicting concentration–dependent pore opening reversal.

EXPERIMENT 5

- Assessment of the Effects of Chloroform Fraction of *Ocimum gratissimum* leaf on Mitochondrial Membrane Permeability Transition pore in the absence and presence of Ca^{2+}.

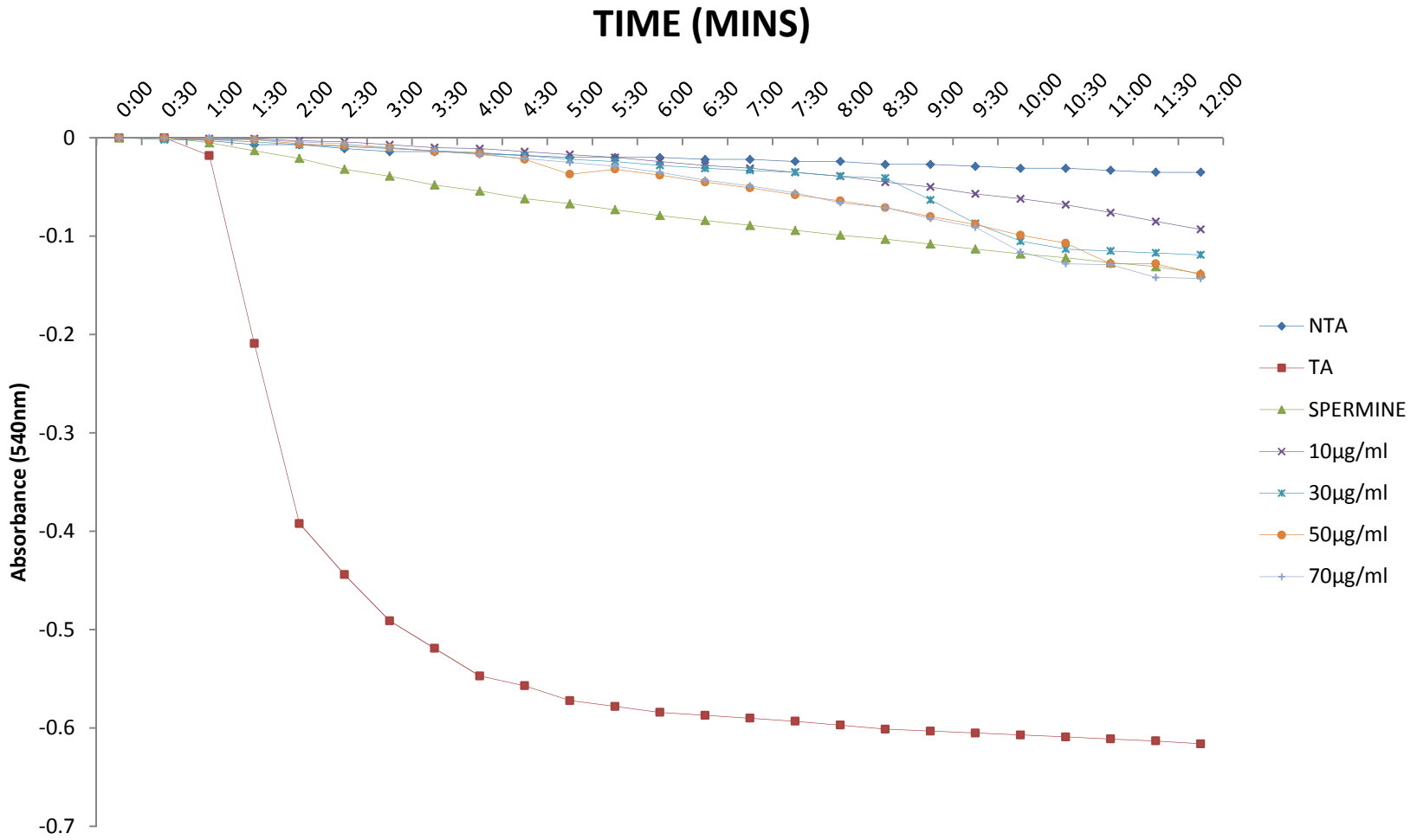

Fig. 7: Assessment of Effect of Chloroform Fraction of *Ocimum gratissimum* Leaf on Mitochondrial Membrane Permeability Transition pore in the absence of Ca^{2+}

Summary

- Fig. 7 illustrates the effect of chloroform fraction of *Ocimum gratissimum* leaves on mitochondrial membrane permeability transition pore in the absence of calcium.
- Mitochondria in the absence of calcium were intact. Significant pore opening was triggered by calcium whereas spermine also reversed the calcium-induced pore opening significantly.
- Treatment of the intact mitochondria with various concentrations (10µg/ml, 30µg/ml, 50µg/ml and 70µg/ml) of chloroform fraction of the plant showed no effect on the mitochondrial membrane as there was no significant difference from the intact mitochondria.

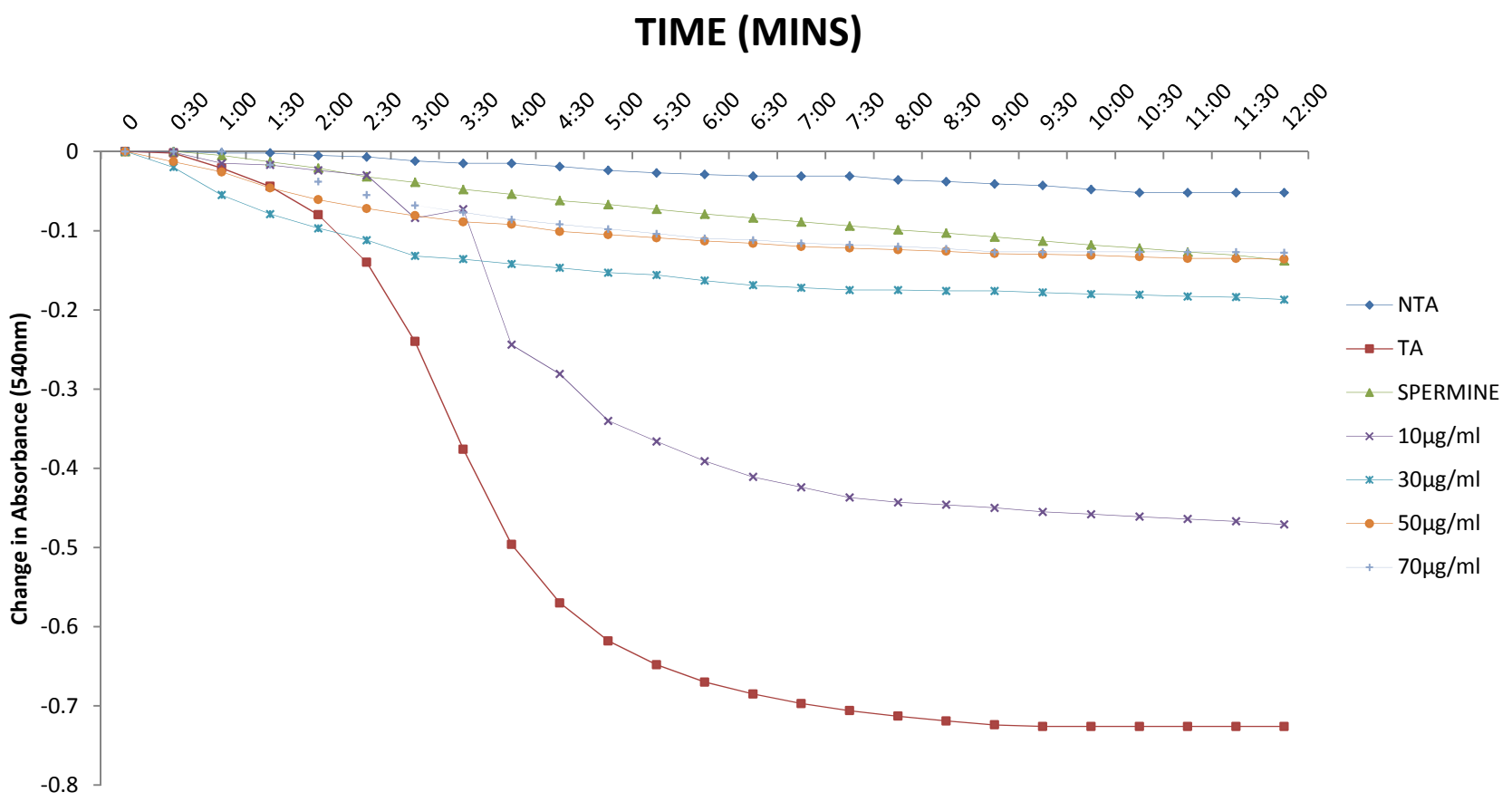

Fig. 8: Assessment of Effect of Chloroform Fraction of *Ocimum gratissimum* Leaf on Mitochondrial Membrane Permeability Transition pore in the presence of Ca^{2+}

Summary

- Fig. 8 shows the effect of chloroform fraction of *Ocimum gratissimum* leaves on calcium-induced opening of MMPT pore.
- The result depicts that there was significant induction of the pore opening triggered by calcium and subsequent reversal of the opening by chloroform fraction of the plant.
- The reversal was 7.65%, 43.4%, 45.7% and 49.3% by 10µg/ml, 30µg/ml, 50µg/ml and 70µg/ml respectively in concentration-dependent manner.

EXPERIMENT 6

- Quantitation of Cytochrome c release by Methanol extract and Chloroform fraction of *Ocimum gratissimum* leaf

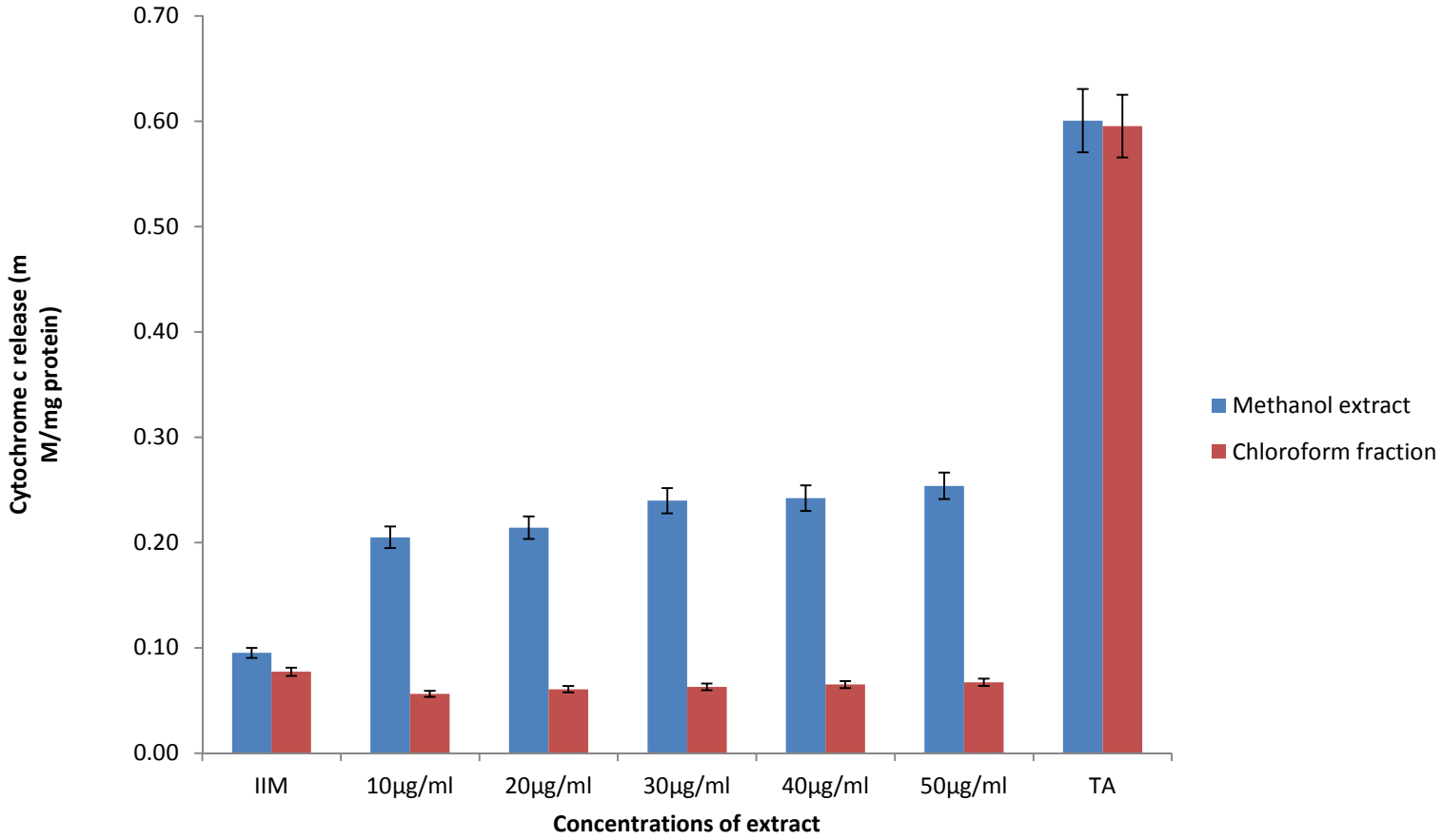

Fig 9: Effect of varying concentrations of methanol extract and chloroform fraction of *Ocimum gratissimum* leaf on Cytochrome C release from MMPT pore

EXPERIMENT 7

- Assessment of the Effects of Incubation of *Ocimum gratissimum* Leaf Extracts on **Fe^{2+}** - induced Lipid peroxidation

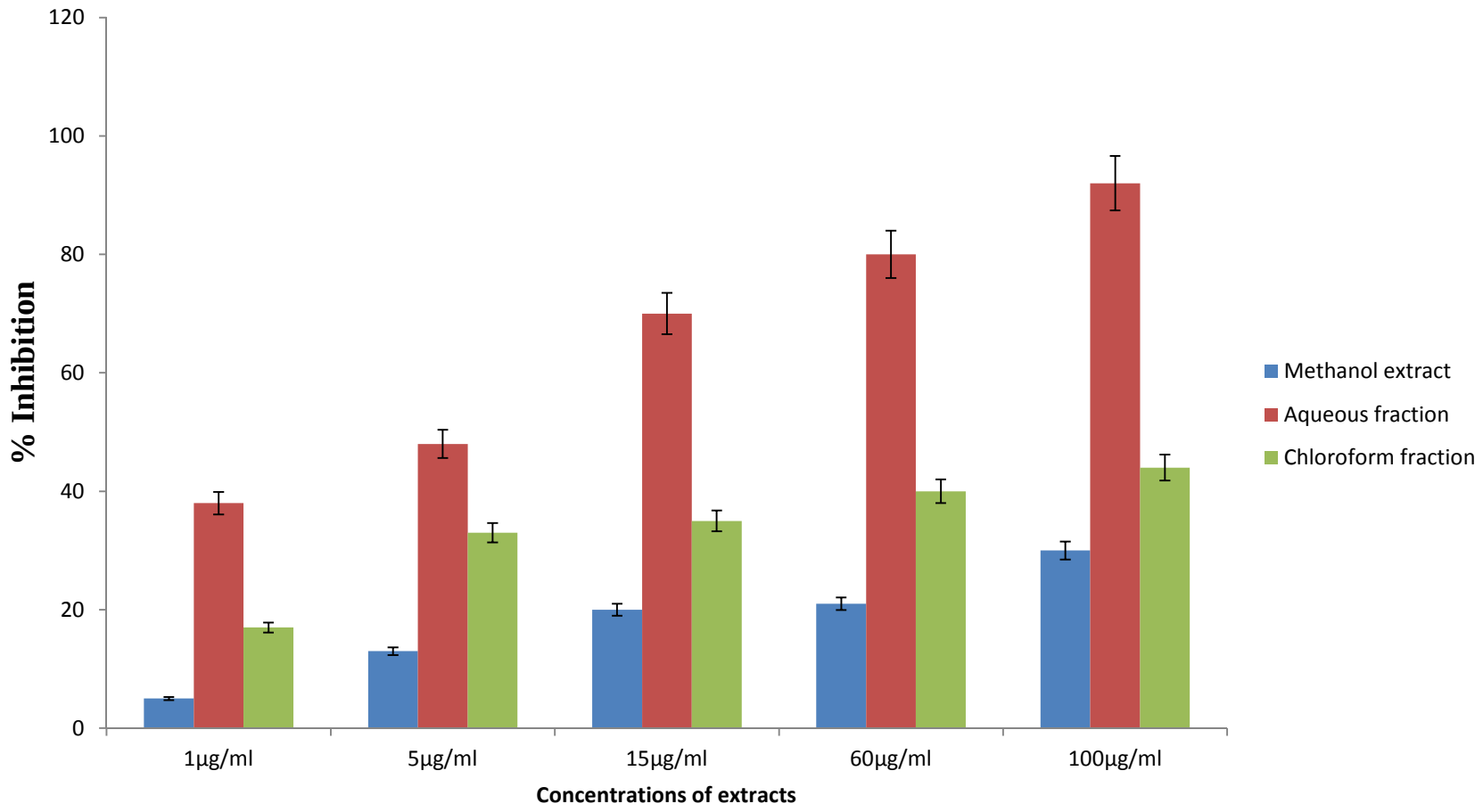

Fig. 10: Effects of *Ocimum gratissimum* leaf extracts on Fe^{2+} -induced lipid peroxidation

Summary

- Fig. 10 shows the effect of *Ocimum gratissimum* leaf extracts on Lipid peroxidation.
- Various concentrations of the plant extracts were found to inhibit lipid peroxidation induced by $FeSO_4$ in concentration-dependent fashion.
- Methanol extract exhibits percentage inhibitions of 5, 13, 20, 21 and 30% at 1, 5, 15, 60 and 100µg/ml respectively.

- Aqueous fraction has percentage inhibitions of 38, 48, 70, 80 and 92% at varying concentrations of 1, 5, 15, 60 and 100µg/ml respectively.
- Whereas chloroform fraction was able to exhibit percentage inhibitions of 5, 33, 35, 40 and 44% at 1, 5, 15, 60 and 100µg/ml respectively.

DISCUSSION

- In the present study, Ca^{2+} was used to induce MPTP opening and there was significant reversal by various extracts of the plant in concentration-dependent manner (crude methanol< chloroform fraction< aqueous fraction), indicating a protection against MPTP opening.

- This effect may be due to the capacity of *Ocimum gratissimum* leaf extracts to counteract the effect of Ca^{2+} and/or ROS, related to its antioxidant activity (Afolabi *et al*., 2007).

- Also, the results clearly demonstrated the efficacy of *Ocimum gratissimum* leaf extracts (crude methanol< chloroform fraction< aqueous fraction) in free radical scavenging as observed in inhibition of lipid peroxidation in rat liver mitochondria.

- Earlier study has indicated that polyphenols effectively block Fe^{2+}-induced TBARS production in mitochondria (Omololu *et al.*, 2011).

CONCLUSION

- The results obtained from this study show that various extracts of *Ocimum gratissimum* leaf have a number of bioactive components which can efficiently scavenge free radicals and thus could be used as anti-ageing, anti-inflammatory and anti-diabetes agents.

- The inhibitory effect on Ca^{2+}-induced MPT pore opening may present *Ocimum gratissimum* as excellent therapeutic agent in conditions where there is upregulated apoptosis as in diabetes mellitus and neurodegenerative diseases.
- However, subsequent research needs to be conducted to ascertain the *in vivo* effect of these fractions on MMPT pore.

REFERENCES

- Hamid A. A, Aiyelaagbe O. O, Usman, L. A. Ameen O. M. and Lawal A. Antioxidants: Its medicinal and pharmacological applications African Journal of Pure and Applied Chemistry Vol. 4(8), pp. 142-151, August 2010.
- Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR, Health benefits of nuts: potential role of antioxidants. Br JNutr. 2006;96(Suppl 2):S52-60.
- Danziel AC, 1996. The useful plants of West Africa. Crown Agents, London, pp 162.
- Effraim ID, Salami HA, Osewa TS (2000). The effect of aqueous leaf extract of *Ocimum gratissimum on haematological and biochemical* parameters in rabbits Afr. J. Biomed. Res. 175-179.
- Afolabi C. Akinmoladun, E. O. Ibukun, Emmanuel Afor, E. M. Obuotor and E.O. Farombi (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum.* Scientific Research and Essay Vol. 2 (5), pp. 163-166.

- Javadov S, Karmazyn M. Mitochondrial permeability transition pore opening as an endpoint to initiate cell death and as a putative target for cardioprotection. Cell Physiol Biochem. 2007;20(1–4):1–22.
- Wu PF, Zhang Z, Wang F, Chen JG. Natural compounds from traditional medicinal herbs in the treatment of cerebral ischemia/reperfusion injury. Acta Pharmacol Sin. 2010;31(12):1523–1531.
- Omololu PA, Rocha JB, Kade IJ. Attachment of rhamnosyl glucoside on quercetin confers potent iron-chelating ability on its antioxidant properties. Exp Toxicol Pathol. 2011;63(3):249–255.
- Kaurinovic B, Popovic M, Vlaisavljevic S, Schwartsova H, Vojinovic-Miloradov M. Antioxidant profile of Trifolium pratense L. Molecules. 2012;17(9):11156–11172.
- Elekofehinti OO, Kade IJ. Aqueous extract of African egg plant inhibit Fe2+ and SNP induced lipid peroxidation in rat's brain- In vitro. Der Pharma Lett. 2012;4(5):1352–1359.

- Mahmoud K, New Hopes and Challenges for Treatment of Neurodegenerative Disorders: Great Opportunities for Young Neuroscientists. Basic and Clinical Neuroscience 2013; 4(1): 3-4.
- Bolanos JP, Moro MA, Lizasoain I, Almeida A. Mitochondria and reactive oxygen and nitrogen species in neurological disorders and stroke: Therapeutic implications. Adv Drug Deliv Rev 2009;61(14): 1299-1315.
- Mohammed A., Tanko Y., Okasha M. A.,Magaji R. A. and Yaro A. H (2007). Effects of aqueous leaves extract of *Ocimum gratissimum* on blood glucose levels of streptozocin-induced diabetic wistar rats. *African Journal of Biotechnology* Vol. 6 (18): 2087-2090.
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951). Protein measurement with Folin phenol reagent. J.Biol. Chem. 193(1): 265-75.

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