Original Research Article

Effect of methanol extract of *Mangifera indica* on mitochondrial membrane permeability transition pore in normal rat liver and monosodium glutamate-induced liver and uterine damage.

ABSTRACT

Introduction: Intrinsically, the mitochondrion has been known to play a crucial role in the induction of apoptosis as a result of the opening of the mitochondrial membrane permeability transition (MMPT) pore which results to the release of cytochrome C and consequently, lead to cell death (apoptosis).

Aim: The aim of this study was to investigate the influence of crude methanol extract of *Mangifera indica* (MEMI) on mitochondrial-mediated apoptosis via induction of MMPT pore opening *in vitro* and *in vivo*

Methods: Mitochondria, isolated from female albino rat liver (between 90-100g), were exposed to varying concentrations (10, 30, 50, 70, and 90µg/ml) of MEMI. Opening of the pore, cytochrome c release, mitochondrial ATPase activity and extent of mitochondrial lipid peroxidation were assessed spectrophotometrically. Histological examinations were also carried out on the liver and uteruses of normal and monosodium glutamate (MSG)-treated rats.

Results: The *in vitro* results showed a significant concentration-dependent induction of pore opening by 1.5, 10.3, 11.5, 13.1 and 17.4 folds, respectively. Oral administration of MEMI at varying doses of 100, 200 and 400mg/kgbwt also showed an induction folds of 0.4, 1.9 and 2.3, respectively, after 14 days, and more significantly, induction folds of 3.4, 6.3 and 15.4, respectively, after 28 days of treatment. Also, MEMI caused a significant release of cytochrome C and enhancement of ATPase activity both *in vitro* and *in vivo* in a concentration and dose-dependent manner. The histological findings also showed that MEMI ameliorated the damage induced in the liver and uterus of MSG-treated rats. It also reduced the MSG-induced uterine hyperplasia in the co-administered group.

Conclusion: These results suggest that MEMI contains bioactive agents that can induce mitochondrial-mediated apoptosis and ameliorate MSG-induced liver damage and uterine hyperplasia. This might be relevant in diseased conditions where apoptosis needs to be upregulated.

Keywords: *Mangifera indica,* Mitochondria Membrane Permeability Transition Pore, Monosodium glutamate

1 INTRODUCTION

Cells are inherently programmed for death in order to maintain cellular homeostasis. Apoptosis, a form of programmed cell death, is one of the most potent defence mechanism by which potentially deleterious and mutated cells are eliminated from an organism while the integrity and architecture of the surrounding tissue is preserved [1]. It plays crucial roles in ensuring proper growth and development, maintenance of tissue homeostasis, differentiation in multicellular organism, metamorphosis and embryogenesis [2]. Experimental evidences have shown that apoptotic processes occur via two major mechanisms: extrinsic or death receptor pathway and intrinsic or mitochondrial pathway [3]. Mitochondria have been seen to play a complex role in apoptosis when biochemical studies identified several mitochondrial proteins that are able to activate cellular apoptotic programs and the emerging interactions between mitochondrial membrane permeability transition pore components [4] via the intrinsic pathway by releasing mitochondrial proteins into the cytosol which normally reside in the intermembrane space [5,6].

The MMPT pore is a protein pore that is formed in the membrane by the opening of a voltage-dependent, high conductance channel (known as the permeability transition pore located in the inner mitochondrial membrane) of mitochondria under certain pathological conditions which include stress, traumatic brain injury and stroke [7]. Studies have shown that the permeabilization of the inner mitochondrial membrane is a major event in the induction of mitochondrial pathway of apoptosis which involve the release of an apoptosis-inducing factor (AIF) and cytochrome C which thereby triggers assembly of the apoptosome leading to activation of procaspase-9 to caspase -9 and downstream cleavage of caspases 3, 6 or 7 and subsequently execution of apoptosis [8,9,10]. This serves as a sine qua non to the initiation of cell death [1,5,6,11,12]. Hence, dysfunctioning of the apoptotic program will result in pathological conditions such as cancer, autoimmune diseases, neurodegenerative disorders, ischemic diseases, etc. [13,14].

Different research works have revealed that some medicinal plants possess phytochemicals e.g. capsaicin in chili pepper, quercetin in onions, resveratrol in grape, organo-sulfur compounds in garlic, and lycopene in tomatoes among many others [15] which exhibit many beneficial effects on human health, targeting mitochondria apoptotic machineries through the activation of MPTP by bioactive agents or natural compounds towards efficient and selective treatment of diseases with too little apoptosis such as cancer [16].

Bioactive agents present in medicinal plants elicit their chemoprotective and therapeutic effects through the modulation of the opening of MMPT pore which could serve as a prima facie target for the design of novel pharmacological drugs [15[. *Mangifera indica* is a species of flowering plants belonging to the family of Anacardiaceae and it is popularly known as mango. The young leaves contain phytochemicals such as tannins, flavonoids, steroids, cardiac glycosides, alkaloids and carbohydrates [17]. Its folkloric use has been reported and dated over 4000 years to be an important herb in the Ayurvedic and indigenous medical

systems. One of the chemical constituents includes Mangiferin which is a polyphenolic antioxidant and a glucosyl xanthone [18]. It has strong antioxidant, wound healing, anti-lipid peroxidation, immunomodulation, cardiotonic, hypotensive, antidegenerative and antidiabetic activities [19] and also used locally in the treatment of asthma, cough, fibroid, curing of wounds, etc [20]. It has also been shown to have anticarcinogenic effects [21]. Additionally, it has been reported to be beneficial in certain disorders connected with women's reproductive organs, treatment of fibroid, wounds, cough, hypertension, rheumatism diarrhea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage, and pile. They also have some antibiotic and anti-inflammatory properties [20].

Monosodium glutamate is a sodium salt of glutamate and it is generally used as a flavor enhancer. Its toxic and deleterious effects on various organs in rat model such as the uterus, ovaries and in tissues have been reported [22,23,24]. Studies have shown that MSG also triggers an increase in the number of cells called hyperplasia that serves as an indication to the presences of uterine tumour, the increase in the level of estrogen and progesterone, which is one of the biomarkers for tumor development in rat model and has also been reported to induce fibroid [23,24,25]. Due to paucity of information of the effect of the plant on mitochondrial membrane permeability transition pore, this led to a pivotal study to investigate the influence of MEMI on rat liver mitochondrial membrane permeability transition pore in normal and monosodium glutamate-treated rats.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS

Two sets of female virgin rats each weighing between 100-120g were obtained from the Preclinical Animal House, Physiology Department, University of Ibadan, Ibadan, Nigeria and were kept at the Biochemistry Department Animal house, University of Ibadan, Ibadan, Nigeria under light-controlled conditions (12h-light/12h-dark cycle) and in well-ventilated plastic cages. The rats were grouped into four groups with eight animals in each and were kept in ventilated cage with 12 hours light/dark cycling and were given food and water *ad libitum*. The rats were acclimatized for two weeks. All experiments have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.2 FIRST SET: The rats were grouped into: Control, 100mg/kg, 200mg/kg and 400mg/kg (bdwt). The varying doses were given based on their body weight. Assays were carried out after 14 and 28 days of treatment. Histological study was also carried out on the liver.

2.3 SECOND SET: The rats were grouped into: Control, MSG only, MSG+MEMI and MEMI only. 200mg/kg of MSG was administered as the toxicant while 100mg/kg of MEMI

was given as the candidate potential drug. The rats were sacrificed after 28 days and histology was carried out on their liver and uteruses.

2.4 MONOSODIUM GLUTAMATE

Ajinomoto (a brand of monosodium glutamate) was bought from Bodija market, Ibadan, Nigeria, at a wholesale distributor. A solution was prepared by dissolving 10g in 20ml of distilled water.

2.5 PLANT MATERIAL

The leaves of *Mangifera indica* were bought from Bode market in Ibadan, Oyo State and authenticated at Botany department, University of Ibadan, Ibadan, Nigeria with voucher number UIH 22555.

2.6 PREPARATION OF EXTRACT

The leaves of *Mangifera indica* were cut into smaller pieces, washed, shade-dried under laboratory conditions for 4 weeks and pulverized to powder using a grinder. It was then soaked in Methanol for 72 hours. The filtrate obtained was concentrated using a Vacuum rotary evaporator (N-100, Eyla, Tokyo, Japan) and was later concentrated to dryness using a water bath at 37^{o}C and the residues was transferred to a bottle and stored in a refrigerator until use.

2.7 REAGENTS

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

2.8 ISOLATION OF RAT LIVER MITOCHONDRIA

Rat liver mitochondria were isolated essentially according to the method of Johnson and Lardy [26] and as modified by Olorunsogo *et al* [27]. The animals were sacrificed by cervical dislocation and the livers excised and trimmed to wash excess tissue. The livers were then weighed, washed with homogenising buffer (210mM Mannitol, 70mM Sucrose, 5mM HEPES-KOH, pH 7.4 and 1mM EGTA), and homogenised as a 10% suspension in ice –cold buffer using a Porter Elvehjem glass homogeniser. 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 (210mM Mannitol, 70mM sucrose, 5mM 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 (210mM Mannitol,70mM sucrose,

5mM HEPES-KOH, pH 7.4), and immediately dispensed into eppendorf tubes and kept on ice.

2.9 MITOCHONDRIAL SWELLING ASSAY

Mitochondrial membrane 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 [28]. Mitochondria (0.4mg protein/ml) were preincubated in the presence of 0.8µM rotenone in a medium containing 210mM mannitol, 70mM sucrose and 5mM HEPES-KOH (pH 7.4) for 3mins at 27^0C prior to the addition of 120µM $CaCl_2$. Thirty seconds later, 5mM succinate was added and mitochondrial permeability transition quantified at 540nm for 12mins at 30secs interval. To test the intactness of the mitochondria, 4mM spermine was added immediately following the addition of rotenone and just before the addition of mitochondrial fraction.

2.10 DETERMINATION OF MITOCHONDRIAL PROTEIN

Mitochondrial protein concentration was determined according to the method of Lowry *et al*. [29] using bovine serum albumin as standard.

2.11 ASSESMENT OF MITOCHONDRIAL FOF1 ATPASE ACTIVITY

FoF1 Adenosine triphosphatase which was determined by the method of Lardy and Wellman as modified by Olorunsogo and Malomo [30]. Each reaction mixture contained 65mM Tris-HCl buffer pH 7.4, 0.5Mm KCl 1Mm ATP and 25Mm sucrose. The reaction mixture was made up to a total volume of 2ml with distilled water. The reaction was started by the addition of mitochondrial suspension and was allowed to proceed for 30 mins at 27^0C. The reaction was stopped by the addition of 1 ml of a 10% solution of sodium dodecyl sulphate. The zero time tube was prepared by adding the solution of ATP to the reaction vessel following the addition of sodium dodecyl sulphate. 2,4 Dinitrophenol (2,4 DNP) was used as a standard uncoupling agent.

2.12 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 [31] and as modified by Olorunsogo and Bababunmi [32]. 300µl of each solution was dispensed into fresh test tubes, followed by the addition of 300µl of distilled water to each of the test tube. To this was added 1 ml of 5%

ammonium molybdate and 1 ml of 9% freshly prepared solution of ascorbic acid. The tube was well mixed and allowed to stand for 20 minutes. The absorbance was read at 680nm. A water blank was used to set the spectrophotometer at zero.

2.13 INHIBITION OF LIPID PEROXIDATION

2.13.1 *In vitro*

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using mitochondria as lipid rich media [33]. Mitochondria (2mg/ml protein) and varying concentrations (100µg/ml – 800µg/ml) of fraction were added to each test tube and made up to 1 ml with distilled water; 0.05 ml of FeSO4 (0.07 M) was added to induce lipid peroxidation and the mixture incubated for 30 min. 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 min. After cooling, 3.0 ml of butan-1-ol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532nm. Percentage inhibition of lipid peroxidation by the extract was calculated as [AC-AE/AC] x 100. Where AC is the absorbance value of the fully oxidized control and AE is the absorbance in the presence of extract.

2.13.2 *In vivo*

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale [34]. Under acidic conditions, malondialdehyde (MDA) produced from the peroxidation of fatty acids reacts with the chromogenic reagent 2-thiobarbituric acid to yield a pink coloured complex with maximum absorbance at 532 nm.

2.14 ASSAY OF 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.*, [35]. Mitochondria (1mg protein/ml) were preincubated in the presence of 0.8µM rotenone in a medium containing 210mM mannitol, 70mM sucrose and 5mM HEPES-KOH (pH 7.4) for 30mins at 27^{o}C in the presence of different concentrations of the fractions, using 24mM 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 414nm which is the soret (γ) peak for cytochrome C.

2.15 Histological Study:

The liver and the uterus were harvested, cleaned of blood and thereafter used for histopathology study. The liver and uterus were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 6 microns thick were obtained using a rotatory microtome. The deparaffinised sections of the liver were stained with hematoxylin and eoxin while the uterus were stained routinely with masson trichome stain. Photomicrographs of the histological results were obtained.

2.16 STATISTICAL ANALYSIS OF DATA

The data were statistically evaluated using one way analysis of variance (ANOVA). All the results were expressed as mean ± standard deviation (SD). The $p < 0.05$ were considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Calcium-induced mitochondrial membrane permeability transition pore opening in normal rat liver mitochondria and its reversal by Spermine (in vitro)

The data presented in Figure 1 shows that there were no significant changes in the volumes of intact mitochondria respiring on succinate in the presence of rotenone over a period of twelve minutes. Upon the addition of calcium, there was a highly significant increase in MMPT pore opening which was almost completely reversed by spermine. This shows that the mitochondria used in this study were not uncoupled and suitable for use.

3.2 Effects of varying concentrations of Methanol Extract of *Mangifera indica* (MEMI) on the MMPT pore in the absence and presence of Ca^{2+}

Figure 2 shows the effect of various concentrations of MEMI on MMPT pore in the absence of calcium. In the absence of Ca^{2+}, the varying concentrations (20, 60, 100, 140 and 180 µg/ml) of MEMI significantly induced pore opening by 1.5, 10.3, 11.5, 13.1 and 17.4 fold respectively, and as shown in figure 3, calcium further potentiated opening of the pore by 10.1, 14.2, 17.4, 22.7 folds respectively.

3.3 Calcium-induced mitochondrial membrane permeability transition pore opening in control rat liver mitochondria and its reversal by Spermine (*in vivo*)

Figure 4 shows a representative profile of intact mitochondria of control animal respiring on succinate in the presence of rotenone over a period of twelve minutes at the end of 14 and 28 days of administration. When calcium was added, there was a highly significant increase in MMPT pore opening and were reversed by spermine. This implies that the mitochondria of the control animal used in this study were intact and suitable for the experiment.

3.4 Effects of varying doses of Methanol Extract of *Mangifera indica* (MEMI) on the MMPT pore after 14 and 28 days of treatment

Figure 5 shows that the varying doses of MEMI (100, 200 and 400mg/kgbw) caused an induction of pore opening by 0.4, 1.9 and 2.3 folds, respectively, at the end of fourteen days of treatment. There was further induction of pore opening by 3.4, 6.3 and 15.4 folds, respectively, after 28 days of treatment as shown in figure 6.

3.5 Effects of methanol extract of *Mangifera indica* on mitochondrial FoF1 ATPase activity (*in vitro* and *in vivo*)

Mitochondrial ATPase activity was enhanced by MEMI in a concentration-dependent manner (25µg/ml, 75µg/ml, 125µg/ml, 175µg/ml and 225µg/ml) with 225 µg/ml having the highest ATPase activity when compared with the control as shown in figure 7. Also, oral administration of MEMI, at varying doses, as shown in figure 8 caused a slight enhancement of mitochondrial ATPase activity at the end of 14 days while there was a more significant enhancement of mitochondrial ATPase activity ($p<0.05$) after 28 days of treatment as shown in figure 9 with the 400mg/kg bdwt having the highest enhancement of ATPase activity

3.6 Effects of varying concentrations of methanol extract of *Mangifera indica* on Cytochrome c release in rat liver Mitochondria

The effect of MEMI on cytochrome C release was depicted in figure 10. There was a significant release of cytochrome C in a concentration-dependent manner. The result showed that on addition of varying concentrations of MEMI to MSH-pre-incubated mitochondria, there was concentration-dependent release of cytochrome c.

3.7 Effects of methanol extract of *Mangifera indica* on lipid peroxidation in normal rat liver mitochondria (*in vitro* and *in vivo*)

Figure 11 shows the effect of varying concentrations (50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml and 800µg/ml) of MEMI on lipid peroxidation. The extract inhibited Fe^{2+}- induced lipid peroxidation in a concentration-dependent manner by 11.9%, 14.1%, 29.3%, 41.4% and 67.2%, respectively, with the highest concentration having the highest inhibitory effect.

Furthermore, as depicted in figure 12, varying doses of MEMI inhibited lipid peroxidation as shown by the amount of malondialdehyde produced with increase in dosage. The highest dosage group (400mg/kg) produced the least malondialdehyde.

3.8 Histological assessment of the effect of MEMI on the liver and uterus of normal and MSG-treated rats

Figures 13a, b, c and d show the photomicrograph of the liver section of rats treated with varying doses (100, 200 and 400mg/kg bdwt) of MEMI. The histological results show that there was no lesion at a lower dose while a toxic effect may be encountered at a higher dose. Figures 14a, b and c show the effect of MEMI on the liver of normal and MSG-treated rats. The results show that there was a severe disseminated periportal infiltration by inflammatory cells in MSG-treated group. The group that received MSG co-administered with MEMI showed a moderate disseminated periportal infiltration by inflammatory cells when compared with the MSG-treated group. Results from the uterus in figures 15a, b, c and d show that the MSG-treated rats had an increase in collagen fibre and also, increase in the number of masson trichome-stained nuclei cells per unit area. The group that received MSG co-administered with MEMI showed a reduction in collagen fibre and also, reduction in the number of masson trichome-stained nuclei cells per unit area while MEMI-treated group showed a normal

histology when compared with the MSG-treated group. These results suggest that MEMI was able to protect against MSG-induced liver damage and also alleviate uterine hyperplasia induced in the MSG- treated rats.

4 DISCUSSION

The mitochondrion is an important organelle and plays a vital role in apoptosis. Apoptosis is a programmed cell death and it is one of mechanism for cellular defence against cancer, because it destroys potentially deleterious and mutated cells (Reed, 1999). Intrinsically, the mitochondrion has been known to play a crucial role in the induction of apoptosis because, the opening of the mitochondrial membrane permeability transition (MMPT) pore results in the release of cytochrome C and other proapoptotic proteins and consequently leads to cell death. The MMPT pore serves as a useful chemotherapeutic strategy for drug development in diseased conditions where the upregulation or downregulation of apoptosis is needed [36,37]. *Mangifera indica* has been reported to contain chemical constituents e.g. mangiferin which is a polyphenolic antioxidant and a glucosyl xanthone. and phytochemicals such as tannins, flavonoids, steroids, cardiac glycosides, alkaloids and carbohydrates. It has strong antioxidant, anti-lipid peroxidation, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic activities [19]. MSG is globally consumed and there has been a major debate as regards its toxic effect when consumed because it has been reported to increase the level of estrogen, which leads to induction of fibroid in female rats [23]. MSG has been reported to increase collagen fibre in the uterus and number of cells termed endometrium hyperplasia. Statistically, it is said that one out of four women over 45 years old in the United States are affected with fibroid tumors [25]. Uterine hyperplasia which occurs as a results of excessive proliferation of the cells of the endometrium or inner lining of the uterus due to imbalance in the high level of estrogen to progesterone can lead to uterine fibroid, endometriosis and endometrium carcinoma. Researchers have found out that up to 50 percent of females are ascertained to develop endometrium hyperplasia even before menopause and this is as a result of increase in estrogen level to progesterone [23]. In this study, the first experiment showed that exogenous calcium which is a potent inducer, caused an amplitude opening in the mitochondria membrane permeability transition pore (MMPT) and in the presence of spermine, there was a reversal of the initial action and confirming the action of spermine as an inhibitor of MMPT pore opening. This therefore shows that the

mitochondria were suitable for use. The varying concentrations (20, 60, 100, 140 and 180µg/ml) of MEMI in the absence of calcium induced pore opening by 1.5, 10.3, 11.5, 13.1 and 17.4 folds respectively, with the highest induction at 180µg/ml. In the presence of calcium, varying concentrations of MEMI further potentiated the opening of the pore by 10.1, 14.2, 17.4, 22.7 and 26.1 folds respectively, with the highest induction at 180µg/ml. The i*n vivo* results also corroborated the findings from the *in vitro* experiment. The effect of varying doses (100, 200 and 400mg/kg) of MEMI on the MMPT pore after 14 days showed that there was a slight induction by 0.4, 1.9 and 2.3 folds, respectively, and at the end of 28 days, there was a more significant induction of pore opening by 3.4, 6.3 and 15.4 folds, respectively. These findings show that methanol extract of *Mangifera indica* induced the opening of the pore and suggests that it contains bioactive agents that can induce mitochondrial-mediated apoptosis. Long exposure to the treatment also enhanced more induction of pore opening as seen from the 14 and 28 days of treatment. The release of inorganic phosphate (Pi) is an indication of uncoupling of phosphorylation in the mitochondrion and this happens during pathological conditions. The enzyme ATP synthase works via proton motive force to couple ADP and Pi to form ATP while in the reverse direction, ATP is hydrolyzed to ADP and Pi. The inorganic phosphate released is used as an index to measure the ATPase activity. MEMI was able to interact with the MMPT pore and ATPase activity was enhanced in a concentration-dependent manner with the highest concentration having the highest ATPase activity. The *in vivo* results also showed that varying doses (100, 200 and 400mg/kg) of MEMI after 14 days of treatment, enhanced ATPase activity and a more significant increase was recorded after 28 days of treatment. The effect of MEMI on mitochondrial lipid peroxidation was examined and the extract was found to elicit a concentration-dependent inhibitory effect on Fe^{2+} induced lipid peroxidation. The results show that at varying concentrations, there was a significant inhibition of lipid peroxidation by 11.9%, 14.1%, 29.3%, 41.4% and 67.3% at 50, 100, 200, 400 and 800µg/ml of MEMI. The results of the *in vivo* study on lipid peroxidation is also in consonants with the *in vitro* experiment. MEMI was found to elicit a dose-dependent reduction in the level of malondialdehyde produced by MEMI. The results showed that varying doses (100, 200 and 400mg/kg) of MEMI inhibited lipid peroxidation in a dose-dependent manner. This study suggests that MEMI possesses free radical scavenging activity that could protect the physicochemical properties of membrane bilayers from free radical-induced damage.

The release of cytochrome C from the intermembrane space is a sine qua non for apoptosis to take place. The result showed that MEMI caused the release of cytochrome C from the mitochondrial intermembrane space into the cytosol in a concentration-dependent manner with the highest release at the concentration of 180µg/ml. The histological results on the effect of varying doses of MEMI on rat liver showed that at a lower dose, MEMI is safe and tolerable while at a higher dose, might be toxic, as it causes marked disseminated microvesicular steatosis, thrombosis, periportal infiltration and disseminated congestion. Histological findings on the liver of MSG-treated rats showed a severe disseminated periportal infiltration by inflammatory cells. The group that received MSG co-administered with MEMI showed a moderate disseminated periportal infiltration by inflammatory cells. This suggests that MEMI contains phytochemicals that can alleviate MSG-induced damage in rat liver. Histological findings from the myometrium of the uterus of MSG-treated rats showed an increase in collagen fibre and increase in the number of stained nuclei cells, while the group that received co-administration with MEMI showed a reduction in collagen fibre and number of stained nuclei cells when compared with the MSG-treated group. These results suggests that MEMI was able to ameliorate the effect of MSG in the treated rats and also cause a reduction in hyperplasia noticed in the uterus of MSG-treated rats.

In conclusion, this study suggests that MEMI contains phytochemicals that can induce mitochondrial-mediated apoptosis via induction of MMPT pore opening which may be relevant in the management and treatment of diseases where there is need for upregulation of apoptosis. Also, its ameliorative effect on MSG-induced rat liver damage and especially, uterine hyperplasia, justifies its folkloric use in the treatment of fibroid. It is also possible that the mechanism by which MEMI ameliorated MSG-induced uterine hyperplasia might be via upregulation of mitochondrial-mediated apoptosis. Though, the chemical nature of substances responsible for the effect shown by MEMI are still unknown, further work is necessary to elucidate and characterize the structure of putative agent(s) present in MEMI and their effect on induction of mitochondrial-mediated apoptosis.

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RESULTS

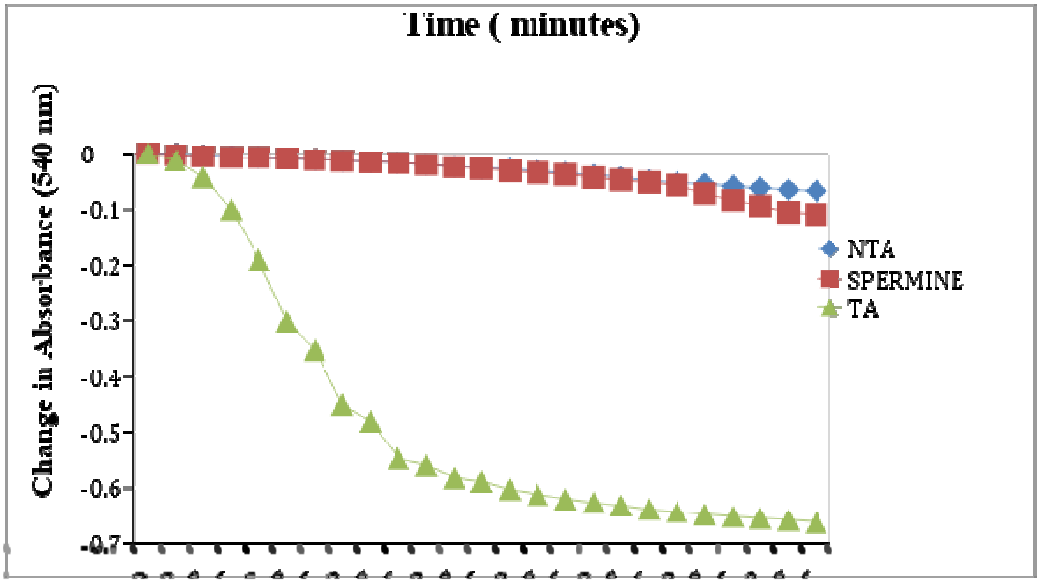

Figure 1: Calcium induced mitochondrial membrane permeability transition pore opening and its reversal by spermine. (*in vitro*)

Abbreviations: NTA-No triggering agent; TA-Triggering agent

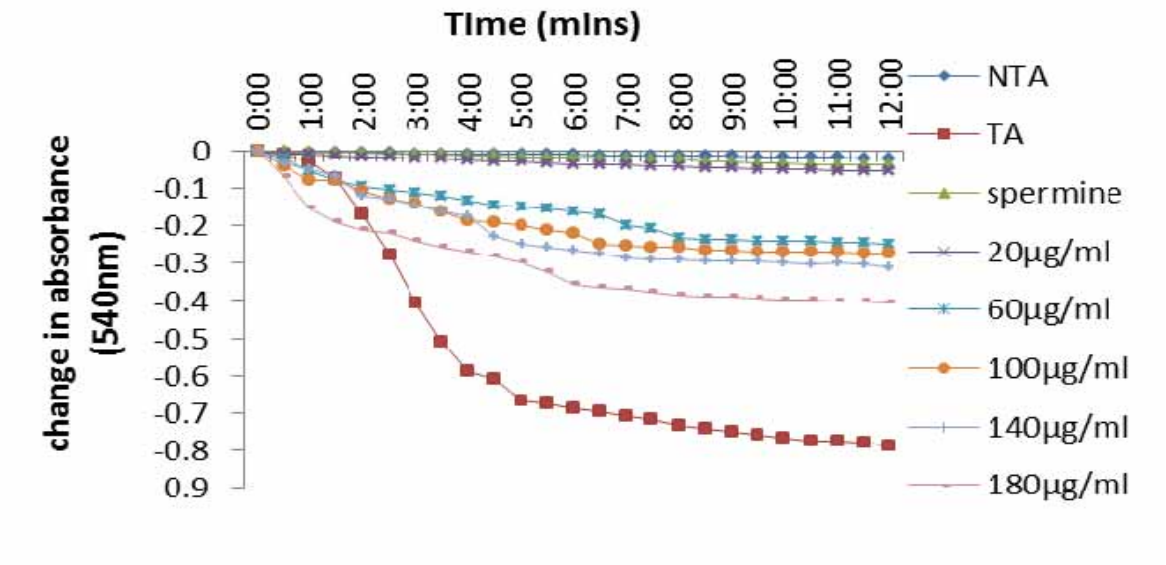

Figure 2: Effect of varying concentrations of MEMI on rat liver mitochondrial membrane permeability transition pore in the absence of calcium.

NTA-No triggering agent TA-Triggering agent

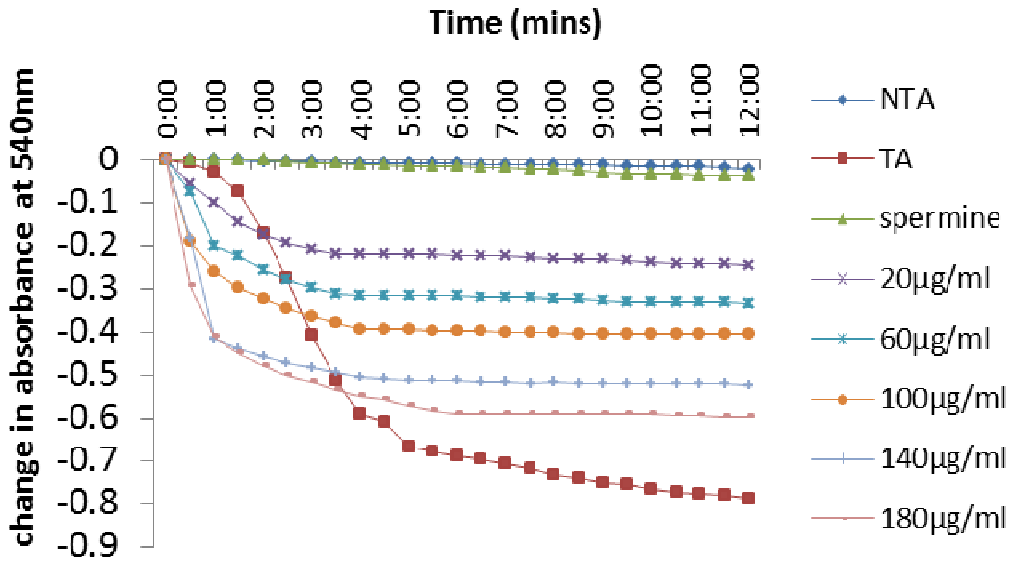

Figure 3: Effect of varying concentrations of MEMI on rat liver mitochondrial membrane permeability transition pore in the presence of calcium.

Abbreviations: NTA-No triggering agent TA-Triggering agent

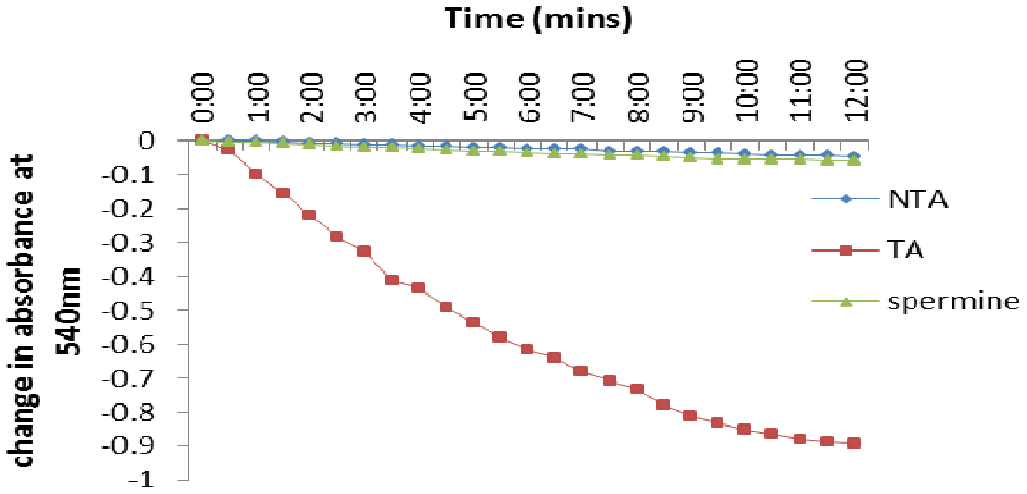

Figure 4: Representative profile of calcium-induced mitochondrial membrane permeability transition pore opening and its reversal by spermine (*in vivo*)

NTA: Non-triggering agent, TA: Triggering agent

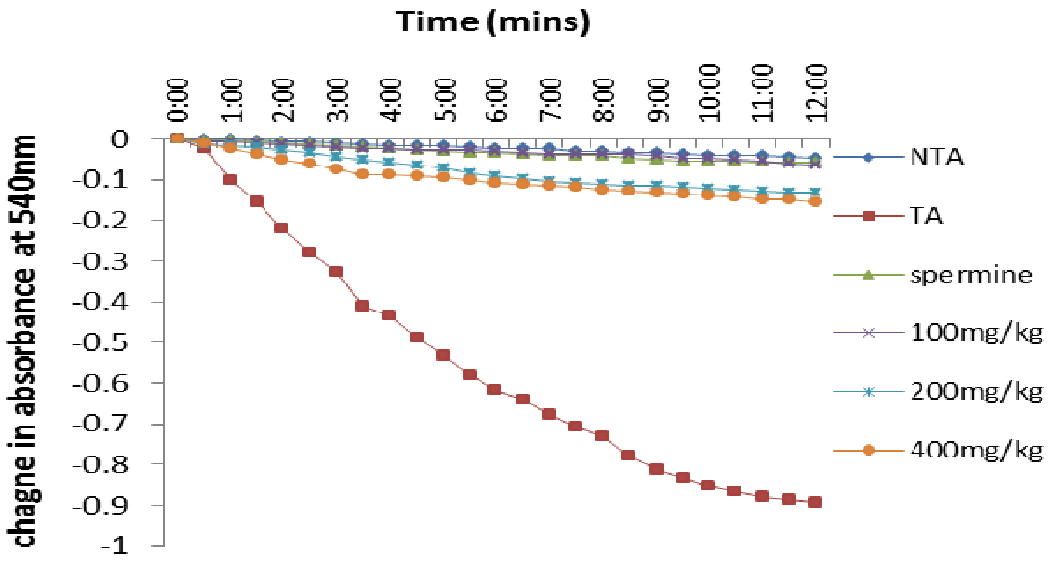

Figure 5: Effect of MEMI on MMPT after 14 days of treatment

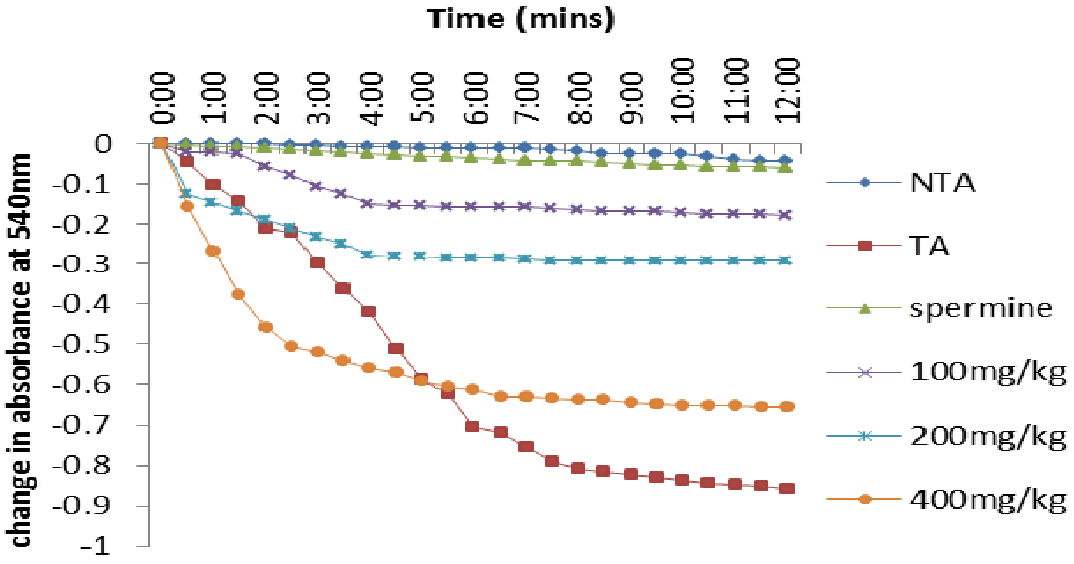

Figure 6: Effect of MEMI on MMPT pore after 28 days of treatment

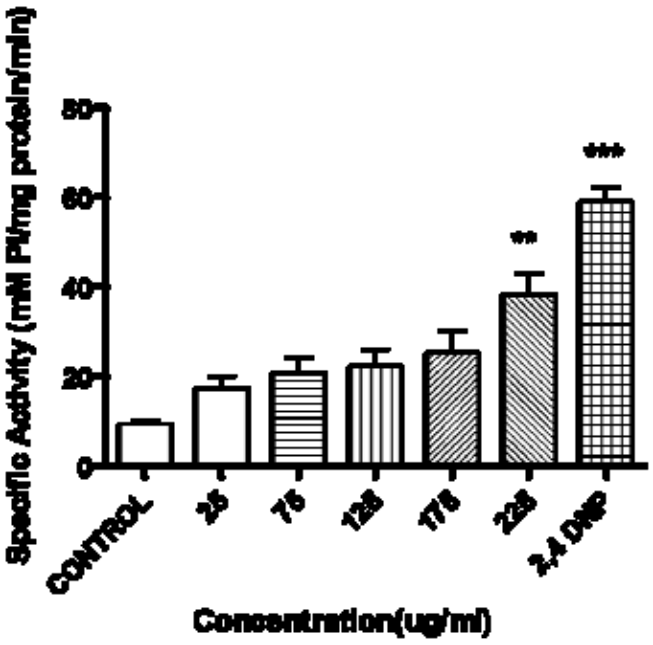

Figure 7: Effect of varying concentrations of MEMI on the mitochondrial ATPase activity. (*in vitro*)

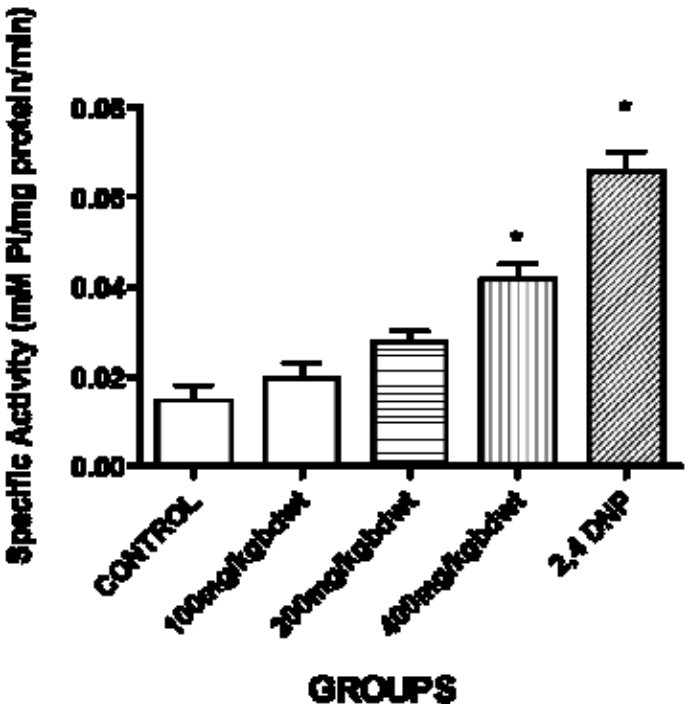

Figure 8: Effects of MEMI on mitochondrial ATPase activity after 14 days of treatment (*in vivo*)

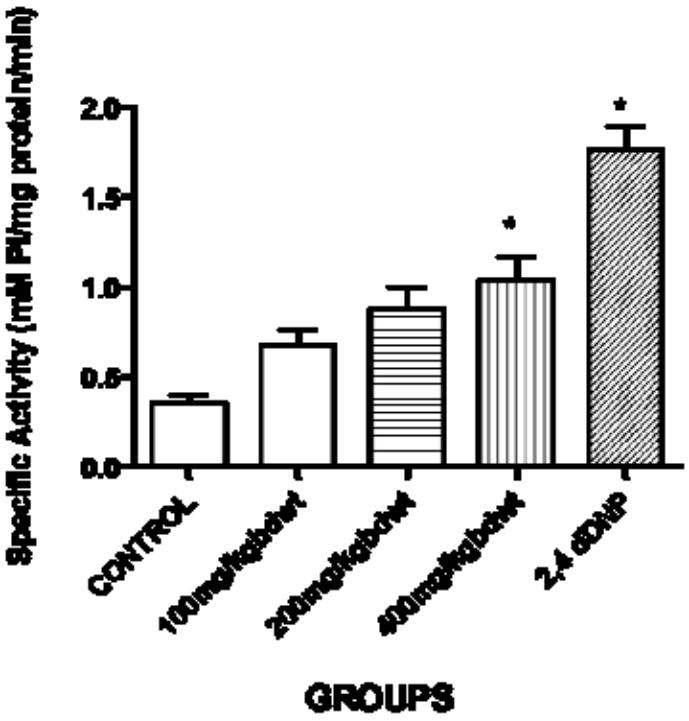

Figure 9: Effects of MEMI on mitochondrial ATPase activity after 28 days of treatment (*in vivo*)

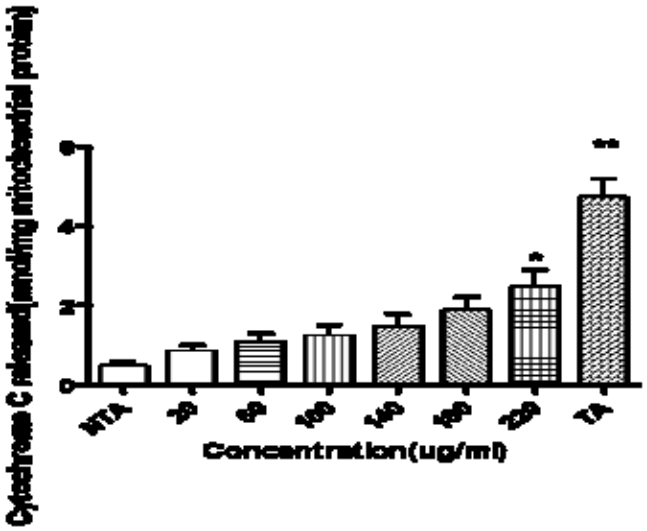

Figure 10: Cytochrome C release by MEMI. (*in vitro*)

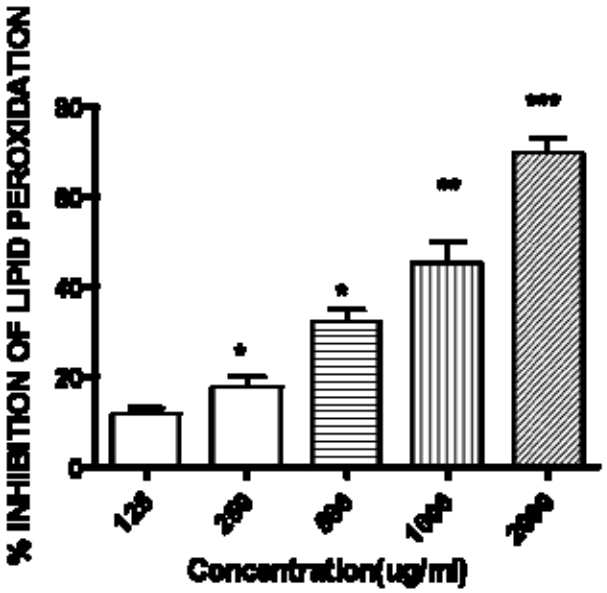

Figure 11: Effects of MEMI on Fe^{2+} -induced lipid peroxidation in normal rat liver mitochondria. (*in virto)*

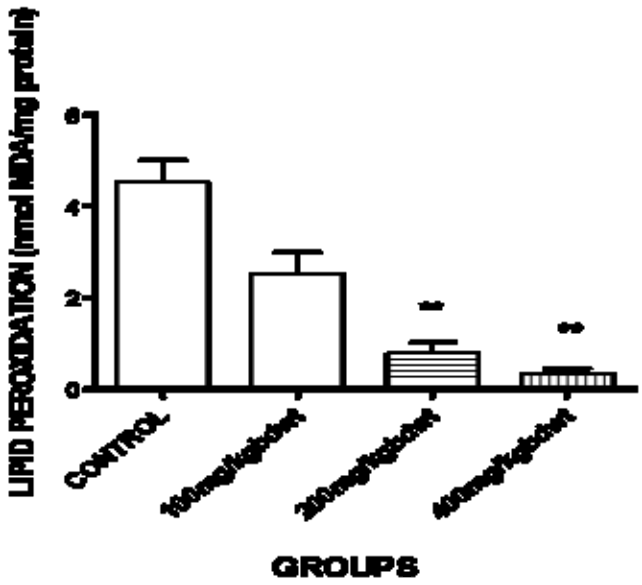

Figure 12: Effects of varying doses of methanol extract of *Mangifera indica* on lipid peroxidation after 28 days of treatment. (*in vivo*)

Figure 13: Photomicrograph of the liver section showing the effect of different doses of MEMI on the hepatocytes (H&E staining)

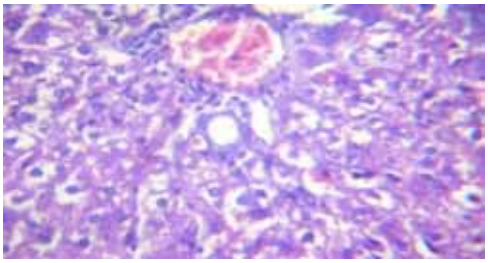

Figure 13a: Control animal showing a normal rat liver morphology (X400)

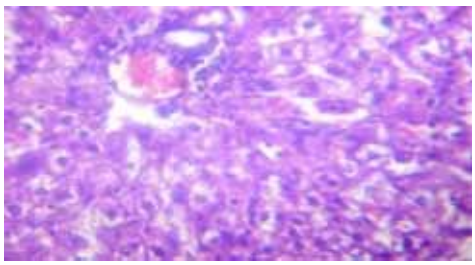

Figure 13b: (100mg/kg) Plate show moderate disseminated microvesicular steatosis (X400)

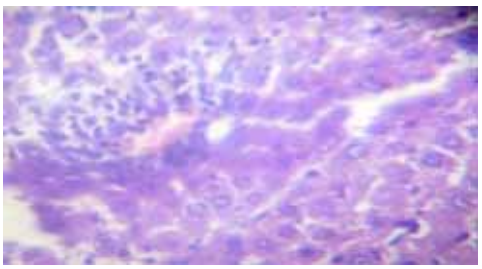

Figure 13c: (200mg/kg): Plate show moderate disseminated microvesicular steatosis and infiltration of zone 2 by inflammatory cells (X400)

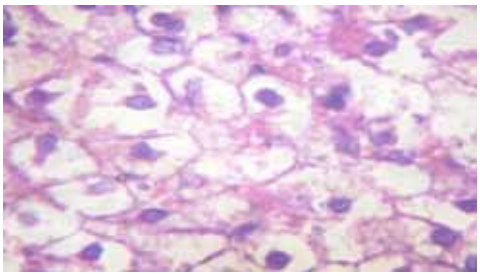

Figure13d: (400mg/kg): Plates show marked disseminated microvesicular steatosis, thrombosis, marked periportal infiltration by inflammatory cells and disseminated congestion

Figure 14: Photomicrograph of the liver section showing the effect of MEMI on normal and MSG-treated rats ((H&E staining)

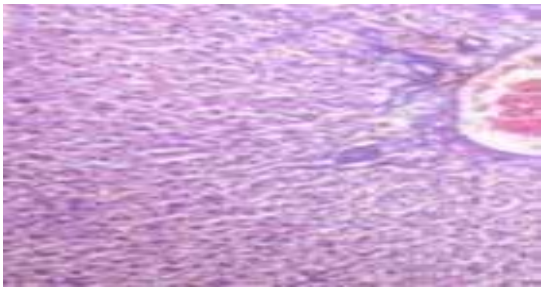

Figure 14a: Section of the control liver showing normal morphology

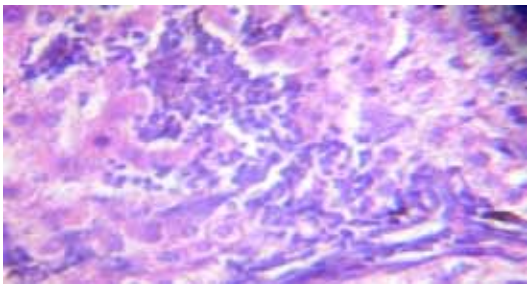

Figure 14b: (MSG): There is severe disseminated periportal infiltration by inflammatory cells, disseminated congestion, multifocal area of thrombosis and focal area of ductal carcinoma.

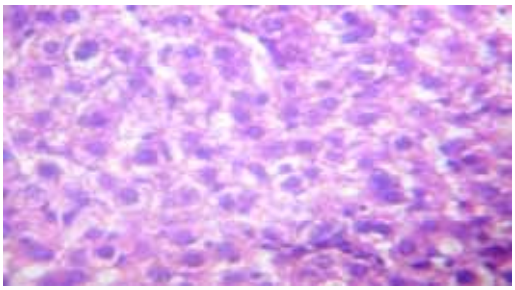

Figure 14c: (MSG+MEMI): Plates show mild disseminated microvesicular steatosis, mild disseminated infiltration of zone 2 by inflammatory cells.

Figure 15: Photomicrograph of the rat uterus (Masson trichrome staining)

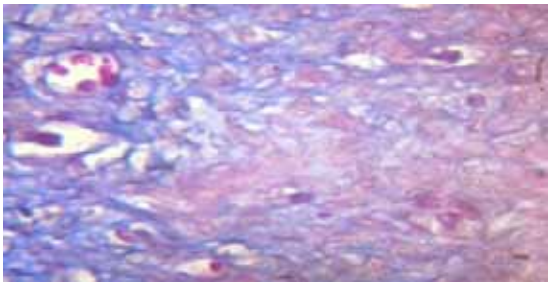

Figure 15a: Control section showing the connective tissue and precursor cells within the endometrial submucosa (Mag. x400).

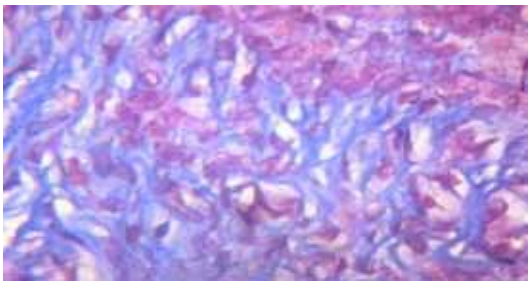

Figure 15b: Treatment section of the myometrium (MSG) showing the connective tissue and precursor cells within the endometrial submucosa (Mag. x400). There is a severe hyperplasia of spindle shaped precursor cells

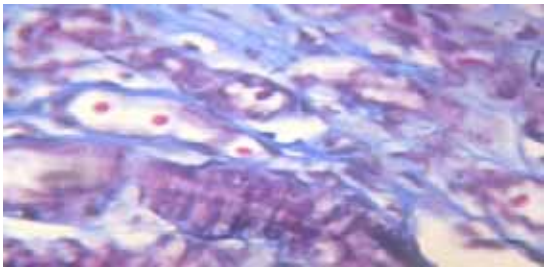

Figure 15c: Treatment section of the myometrium (MSG & MEMI) showing a reduction of hyperplasia compared with MSG-treated group (Mag x400)

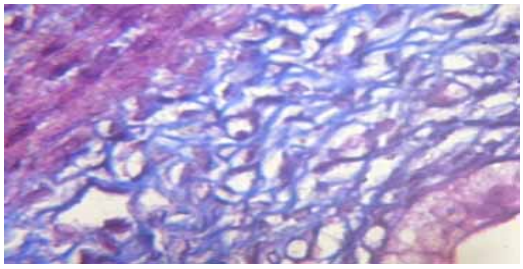

Figure 15d: Treatment section of the myometrium (MEMI) showing the connective tissue and precursor cells within the endometrial submucosa . (Mag. x400)