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Original Article

Jatonik polyherbal mixture induced rat liver MMPT pore opening in normal Wistar rat: *In vitro* and *in vivo* studies

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ABSTRACT

Objective: To assess acute toxicity, the *in vitro* and *in vivo* effects of methanol and ethyl acetate extracts (JME and JEE) of Jatonik polyherbal mixture on some mitochondria-related parameters and their effect on the activity of some liver enzymes.

Methods: Acute toxicity of JME and JEE was determined using Lorke's method. *In vitro* and *in vivo* opening of the mitochondrial membrane permeability transition pore (MMPT pore) was spectrophotometrically assayed. Production of malondialdehyde (MDA) as an index of lipid peroxidation and the activity of mitochondrial ATPase was evaluated *in vitro* and *in vivo* and the effect of JME and JEE on the activity of liver enzymes such as alkaline phosphatase (ALP), aspartate and alanine aminotransferase (AST and ALT) and gamma-glutamyl transferase (GGT) was also investigated.

Results: JME had an LD_{50} of 3 808 mg/kg b.w whereas JEE had an LD_{50} greater than 5 000 mg/kg b.w. of rats. After the rats have been fed with both extracts, a photomicrograph of a piece of liver tissue showed no apparent symptoms of toxicity. From the *in vitro* and *in vivo* studies, both extracts prompted intact mitochondria to open their MMPT pores. When compared to the control, lipid peroxide product release and ATPase activity were significantly increased ($P < 0.05$) *in vitro* and *in vivo*. The activities of AST, ALT, and GGT were all reduced at 50 mg/kg when treated with JME, but the activity of AST was considerably enhanced when treated with JEE ($P < 0.05$). The results revealed that both JME and JEE of the Jatonik polyherbal mixture had low toxicity, profound MMPTpore induction, and enhanced ATPase activity, but an increased MDA production.

Conclusion: Jatonik extracts may be a promising target for drug development in diseases where there is dysregulation of apoptosis, however, further studies are needed to better clarify the molecular mechanism involved in these phenomena.

1. Introduction

Cell death is an important biological process that can be induced by intrinsic or extrinsic apoptosis, and often time abnormalities in its pathway can contribute to pathologies in human disease (Lin et al., 2014). Mitochondria is a dynamic organelle that produces ATP which is also an important contributor to cell activities in both development and cell death processes. Mitochondrial permeability transition pore (mPTP) regulates this dual function of mitochondria (Pérez & Quintanilla, 2017) and hence its involvement in apoptotic cell death via the formation of a nonspecific pore formed from a voltage–dependent anionic channel (VDAC), the adenine nucleotide translocase and cyclophilin-D (cyP-D) at contact sites between mitochondrial outer and inner membranes (Crompton, 1999). Mitochondria permeability transition (mPT) which is mediated by the mitochondrial permeability transition pore (mPTP) causes the permeabilization of the outer membrane of the mitochondria (Bonora et al., 2021) and the opening of a nonspecific pore in the inner membrane of the mitochondria, converting them from organelles whose production of ATP sustains the cell to instruments of death (Halestrap, 1999). Opening of the mPTP can be triggered by calcium overload that was stimulated by mitochondria metabolism (Zhou et al., 2022) and this allows the release

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of intermembrane space proteins such as cytochrome C and other proapoptotic molecules and it is thought to be a point of no return for cell death (Dadsena et al., 2021). Hence, stimulation of the mitochondrial membrane permeability transition (MMPT) pore has been linked to the apoptosis (programmed cell death) cascade of events (Adegbite et al., 2015).

Nature is a source of anticancer drugs with an abundant pool of diverse chemicals and pharmacologically active compounds (Sharifi-Rad et al., 2019). Natural plant products with potent and apoptosis induction properties are extensively being investigated for their cancer chemopreventive potential (Badmus et al., 2015). According to their ancestral use in traditional medicine, various plant extracts have been shown to activate apoptosis in human cancer cells (Rajabi et al., 2021). Hence, our interest was in Jatonik polyherbal mixture.

Jatonik polyherbal mixture consists of *Hunteria umbellata* (K. Schum.) Hallier f. (seed), *Xylopia aethiopica* (Dunal) A. Rich. (fruit), *Lepidium meyenii* Walp. (root) and *Ageratum conyzoides* Linn. (leaves) which are widely used to treat several types of diseases. *H. umbellata* is a member of the family Apocynaceae (Ajiboye et al., 2017) and is locally called osu (Edo), abeere (Yoruba), and nkpokiri (Igbe et al., 2009). It has been reported to contain alkaloids, saponins, and steroids (Falodun et al., 2006). The seeds, leaves, and roots are used as decoctions in the Western and Southern parts of Nigeria for the treatment of blood ailments (Okolafor & Ekhaise, 2021). And it has also been reported for its antimicrobial (Udinyiwe and Aghedo, 2022), antioxidant (Falodun et al., 2006), anti-inflammatory (Adeyemi et al., 2011) and analgesic uses (Igbe et al., 2010). *A. conyzoides* is known as Billy goat weed and belongs to the family of Asteraceae (Burkill, 1985). It is an annual herb with compounds including alkaloids, flavonoids, and terpenoids (Okunade, 2002). It has been reported to have antioxidant (Adetuyi et al., 2018), antiradical and anticancer activities (Adebayo et al., 2010). *L. meyenii* (Maca) has antioxidant and antitumor activities (Fu, Wei, Gao, & Chen, 2021). Maca contains some effective antioxidants, a high percentage of glucosinolates, polyphenols (flavonolignans), and a high antioxidant capacity (Tafuri et al., 2019). Various bioactivities of maca include enhanced reproductive health, antifatigue, neuroprotection, antimicrobial activity and anticancer (Wang & Zhu, 2019). On the other hand, *X. aethiopica* also known as Ethiopian pepper has great medicinal uses and has been reported to be used in the treatment of fibroid, dysentery and cough (Fetse et al., 2016). Although some of these plants have been reported to have anticancer properties because of the presence of phytochemicals present in them, none have been tested either alone or in synergy with other plants as a mixture for its ability to cause apoptosis via the opening of mPTP. The synergistic effect of medicinal plants used in polyherbal preparations leads to a better therapeutic outcome by increasing their protective effects while decreasing their toxicity (Han et al., 2019; Madić et al., 2019).

2. Materials and methods

2.1. Collection of components and preparation of polyherbal mixture

The polyherbal mixture was purchased at Jawesola store, Oja Titun, Ogbomoso, Nigeria. *H. umbellatae* (seeds), *X. aethiopica* (fruits), *L. meyenii* (roots) and *A. conyzoides* (leaves) were the constituents of the polyherbal mixture used in this study. The plants were authenticated at the Ladoke Akintola University of Technology Herbarium by Prof. A.T.J. Ogunkunle and voucher numbers LHO 632, LHO 633, LHO 634 and LHO 635 respectively were obtained for them. The method of Alade and Irobi (1993) with slight modifications was used to extract methanol and ethylacetate from the Jatonik polyherbal mixture. In a nutshell, 200 g of the Jatonik herbal mixture was soaked for 72 h in 2 L of 95% methanol with periodic shaking. The extract was filtered and concentrated at 40 °C using a separating funnel. The extracts' dried residue was kept on a bench until they were needed again. And as needed, stock solutions of 5 g/100 mL were prepared.

2.2. Chemicals and reagents

Mannitol, sucrose, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), ethylene glycol tetraacetic acid (EGTA), spermine, rotenone, sodium succinate hexahydrate, bovine serum albumin (BSA), methanol, sucrose were products of Sigma Aldrich Co., Ltd. (St. Louis, MO, USA). Sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), calcium chloride ($CaCl_2$), potassium hydroxide (KOH), ethanol and methanol were products of BDH Poole Co., Ltd. (England, UK). All chemicals were of analytical grade.

2.3. Experimental animals

The study used Wistar strain male albino rats weighing between 180–200 g procured from LAUTECH Teaching Hospital. We employed 15 animals for the median lethality test, 10 animals for the *in vitro* MMPT assay, and 28 animals for the *in vivo* MMPT experiment. At the animal house of the Department of Biochemistry, LAUTECH, Ogbomoso, they were kept in well-ventilated plastic cages with a controlled light cycle (12 h light/12 h dark) and provided commercial rat chow and clean water ad libitum. They were then acclimatized for two weeks and cared for according to the recommendations of the Faculty of Basic Medical Sciences Animal Care and Use Committee for proper laboratory animal care and ethical approval number FBMS/AEC/BCH/0151/24 was given. Animals in the test groups were given varying amounts of the extract orally for 28 d based on their body weights.

2.4. Acute toxicity study

Lorke's (Lorke, 1983) technique was used for this test. For this test, there were two phases, the initial phase necessitated the use of nine animals. Three groups of three animals (n = 3) were formed from the nine animals. Each group of animals received different doses of the test substance (10, 100, and 1 000 mg/kg). The animals were kept under observation for 24 h to see how they behaved and whether they died. Only three animals were used in the second phase, which was divided into three groups of one animal each. The rats were given greater dosages of both methanol and ethylacetate extract of Jatonik herbal mixture (1 600, 2 900, and 5 000 mg/kg) and then watched for 24 h for behavior and death.

The LD_{50} was calculated following the method described by Lorke (Lorke, 1983). Geometric mean of the maximal dose without mortality × Geometric mean of the minimal dose with mortality = X mg/kg.

2.5. Histological examination

A small portion of the excised liver was preserved in 10% (volume percentage) formalin and processed using standard methods for histological examination. The fixed liver was embedded in a paraffin block and then placed on glass slides. Hematoxylin-eosin (HE) was used to stain the liver section and it was examined using the light microscope.

2.6. Mitochondrial fraction isolation

The low ionic strength mitochondrion was isolated using the modified method of Johnson and Lardy (1967) as reported by Olorunsogo et al. (2021). The animals not fed overnight were sacrificed by cervical dislocation and dissected quickly. The liver was rapidly excised, trimmed to remove excess tissue, and washed in a buffer consisting of 210 mmol/L mannitol, 70 mmol/L sucrose, 5 mmol/L HEPES, 1 mol/L KOH, and 1 mmol/L EGTA (pH 7.4). Thereafter, the excised liver was weighed, chopped, and suspended in the same buffer to make a 10% homogenate. Immediately, the suspension was homogenized on ice using a Teflon homogenizer. The homogenate was sedimented twice at 2 500 rpm for 5 min to remove the nuclear fractions and cellular debris, the supernatant obtained was centrifuged at 13 000 rpm (10 000 g) for 10 min and the mitochondria fraction obtained was washed three times at 12 000 rpm (8 000 g) for 10 min with buffer containing 210 mmol/L mannitol, 70 mmol/L sucrose, 5 mmol/L HEPES, 1 mol/L KOH and 1 mmol/L EGTA (pH 7.4). The mitochondria were immediately suspended in a buffer containing 210 mmol/L mannitol, 70 mmol/L sucrose, and 5 mmol/L HEPES-KOH, (pH 7.4) and then dispensed into 1 mL Eppendorf tube and used fresh.

2.7. Mitochondrial protein determination

Mitochondrial protein was determined according to the method described by Lowry et al. (1951) using BSA as standard.

2.8. Mitochondrial swelling assay (preincubation)

Mitochondria (0.3 mg of protein/mL) was pre-incubated in a 2 cm glass cuvette in the presence of rotenone in mannittol, sucrose and hepes (MSH) swelling buffer (210 mmol/L mannitol, 70 mmol/L sucrose, 5 mmol/L HEPES-KOH, pH 7.4) for about 3 min at 30 °C after which calcium the triggering agent was added 30 s later, and then 5 mmol/L succinate was added to energize the medium. For spermine inhibition and the effect of the extract, spermine and both methanol and ethyl acetate extracts (JME and JEE) of Jatonik herbal mixture were added respectively in each case immediately after the addition of rotenone and the mixture was incubated for 3 min by shaking and thorough mixing. $CaCl_2$, the triggering agent was added immediately after the preincubation and 30 s before the addition of succinate. Varying concentrations of the extracts were used in determining the effect of the Jatonik herbal mixture on mitochondria swelling. The swelling rate was quantified as ΔA_{540}/min/mg. Data reported were representative of multiple ($\geq$3) experiments. (Lapidus & Sokolove, 1993).

2.9. Mitochondrial swelling assay (incubation with calcium)

Mitochondria was incubated in a buffer containing 0.35 g of HEPES, 9.55 g of mannitol, 6.58 g of sucrose and 1.25 g of BSA (pH = 7.4) for around 3 min at 30 °C in the presence of rotenone and calcium (triggering agent) 30 s later, 5 mmol/L succinate was added. For spermine inhibition and the effect of extract respectively, spermine and the methanolic extract were added just after the 3 min incubation of the mitochondria in the presence of rotenone, $CaCl_2$ and 5 mmol/L succinate was added 30 s after the addition of extract.

2.10. Determination of in vitro and in vivo ATPase activity

This was performed according to the method described by Bassir (1963) with slight modifications. The mixture (1 mL) was taken from each of the test tubes and put into a duplicate set of test tubes. A total of 1 mL of freshly prepared ammonium molybdate (1.25%) and 9% ascorbic acid solution (freshly prepared) were added at 30 s intervals. The mixture was mixed and allowed to stand for 30 min after which the absorbance was read using a spectrophotometer at 660 nm.

2.11. Determination of in vitro lipid peroxidation

The extent of lipid peroxide formed was measured by using low ionic strength mitochondrion as a lipid-rich medium according to modified methods of Ohkowa and Ruberto and also reported elsewhere (Ohkawa et al., 1979; Ruberto et al., 2000). Briefly, 0.5 mL of mitochondria suspension and varying concentrations of both JME and JEE of the Jatonik herbal mixture were added to test tubes and made up to 1 mL with distilled water. Lipid peroxidation was induced by the addition of ferrous sulfate (0.07 mmol/L). Furthermore, acetic acid (20%), thiobarbituric acid (TBA, 0.8%), and sodium dodecyl sulfate (SDS, 1.1%) were added to the solution. The mixture was vortexed and heated at 95 °C for 60 min. After cooling the absorbance was measured at 532 nm. All experiments were carried out in triplicates.

2.12. Determination of in vivo lipid peroxidation

The method described by Ruberto et al. (2000) was used for measuring mitochondrial lipid peroxidation. Briefly, 1 mg/mL mitochondria homogenate and varying volumes of known concentrations of the extracts were added to test tubes and made up to 1 mL with distilled water, 0.07 mmol/L ferrous sulfate was used to induce lipid peroxidation, 20% acetic acid, 0.8% thiobarbituric acid (TBA), and 1.1% sodium dodecyl sulfate (SDS) were added, and the resulting mixture was allowed to cool, 5 mL butanol was added to each test tube, which was then vortexed extensively and centrifuged at 4 000 rpm for 10 min. At 532 nm, the upper organic layer's absorbance was measured.

The level of malondialdehyde (MDA), a biomarker of lipid peroxidation, is calculated using the following formula:

$$\text{MDA}\,(\text{units/mg of protein}) = \frac{\text{Absorbance} \times \text{volume of mixture}}{E_{532} \times \text{volume of sample} \times \text{mg of protein}}$$

2.13. Enzyme assays

Randox kits (Randox Laboratories, Crumlin, UK) were used to determine the activities of AST, ALT, ALP, and GGT enzymes.

2.14. Statistical analysis

Results were analyzed using student's *t*-test and one-way ANOVA followed by Dunnett's posthoc test for comparison between control and test groups. All data were expressed as mean ± standard error of the mean. $P < 0.05$ was considered significant. GraphPad Prism 8.01.244 and Microsoft Excel 2016 were used for the analyses.

3. Results

3.1. Histopathologic evaluation

Fig. 1a–f showed the toxicity effect of JME and JEE of Jatonik polyherbal mixture. Histopathology examination of the liver as shown in the control and the test groups showed mild changes. The rats fed with 1 600 and 2 900 mg/kg dosages of JME displayed moderate and marked disseminated microvesicular steatosis respectively with both also showing mild disseminated infiltration of zone 2 by inflammatory cells as shown in Fig. 1b and c, but when

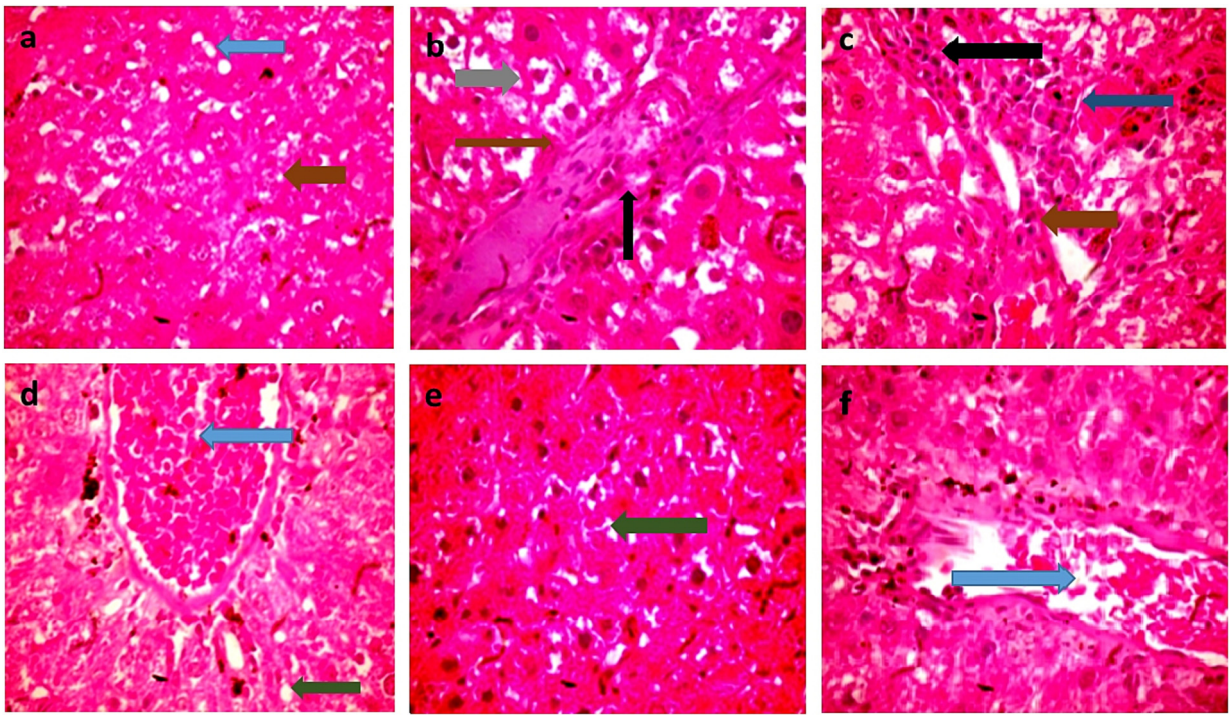

Fig. 1. (a) Photomicrograph of the control group showing mild disseminated infiltration of zone 2 by inflammatory cells. (400×) (b) Photomicrograph of the group treated with 1 600 mg/kg of JME showing moderate disseminated microvesicular steatosis (black arrow), and mild disseminated infiltration of zone 2 by inflammatory cells (brown and grey arrow) (400×). (c) Photomicrograph of the group treated with 2 900 mg/kg of methanol extracts of JME showing marked disseminated microvesicular steatosis (black arrow) mild disseminated periportal infiltration by inflammatory cells (blue arrow), and mild disseminated infiltration of zone 2 by inflammatory cells (brown arrow) (400×). (d) Photomicrograph of the section of the liver tissue of the group treated with 5 000 mg/kg of JME showing disseminated congestion (blue arrow) and moderate disseminated microvesicular steatosis (green arrow) (400×). (e) Photomicrograph of the section of the liver tissue treated with 1 600 mg/kg of JEE. Plates show disseminated congestion, moderate disseminated microvesicular steatosis (green arrow) (400×). (f) Photomicrograph of the group treated with 1 600 mg/kg (400×) of JEE showing mild disseminated infiltration of zone 2 by inflammatory cells (blue arrow).

fed with a high dosage of 5 000 mg/kg, disseminated congestion was shown (Figs. 1d). But in contrast, rats fed with JEE at a low dose of 1 600 mg/kg showed disseminated congestion, moderate disseminated microvesicular steatosis and mild disseminated infiltration of zone 2 by inflammatory as shown in Fig. 1e and f. The damages observed in comparison to control were mild and can be due to several aetiological factors.

The LD_{50} of JME extract was estimated to be 3 808 mg/kg BW. The LD_{50} of rats fed with JEE was greater than 5 000 mg/kg body weight of the rats. The rats were generally active and showed no visible signs of illness or toxicity.

3.2. In vitro and in vivo opening of MMPT pore by JME and JEE

Fig. 2A showed the effect of Ca^{2+} and spermine on the isolated mitochondria of normal rats by preincubating mitochondria in MSH (Mannitol- Sucrose-Hepes) buffer for 3 min before the addition of Ca^{2+}. Changes in absorbance over 12 min showed that Ca^{2+} as an inducer of MMPT pore opening by 305 % and spermine was observed to reverse the opening. Fig. 2B however showed the effect of Ca^{2+} and spermine on isolated mitochondria by incubating with calcium for 3 min. This showed MMPT pore opening by 297 % and it was also reversed by spermine. Fig. 2C and D showed *in vitro* induction of MMPT pore opening by changes in absorbance after the addition of varying concentrations of JME and JEE respectively for a period of 12 min in the absence of calcium (triggering agent). After exposing the mitochondria to varying concentrations (4, 8, 12, 16 and 20 μg/mL) of JME and JEE, MMPT pore opening was triggered by 6.7, 6.7, 6.5, 6.2, 5.6 and 3.4, 3.0, 3.7, 2.5, 4.9 folds, respectively. The effect of the induction was concentration dependent with the lowest concentration having the highest induction of the pore except for JME which has the highest induction of the pore at the highest concentration. The observed pore opening was inhibited by spermine (a standard inhibitor of the pore). The MMPT pore opening at all concentrations was observed to be more than that of Ca^{2+} (a standard triggering agent).

Fig. 3A presented the *in vivo* effect of Ca^{2+} and spermine on isolated mitochondria and changes in absorbance over a period of 12 min. Ca^{2+} triggered the opening of the MMPT pore and the observed opening was reversed by spermine. Fig. 3B and C showed the effect of various concentrations (30, 40, 50 mg/kg) of JME and JEE in the absence of Ca^{2+} *in vivo*. At all concentrations, the opening of the pore was triggered. For the JME-treated rats (Fig. 3B), the opening of the pore was observed to be 3.7, 1.4 and 2.2 folds, respectively. This result showed that the extract was more potent at opening the pore at the lowest concentration and even more potent at the highest concentration. Treatment with the extract was observed to have an inductive effect of MMPT pore opening greater than that of Ca^{2+} (standard trigger of the pore). Fig. 3C showed the effect displayed in the JEE-treated rats, opening of the MMPT pore was also triggered at all concentrations but the triggering elicited was lower when compared with Ca^{2+} induced opening. This result could present JME as a better agent of MMPT pore opening.

3.3. Effects of JME and JEE on rat liver mitochondrial ATPase

Fig. 4A depicted the *in vitro* effect of different doses of Jatonik methanol and ethylacetate extracts (JME and JEE) on rat liver mitochondrial ATPase (mATPase) activity (pH 7.4). The results revealed that JME-treated rats significantly (P < 0.01, 0.001) boosted the activity of mATPase in a concentration-dependent way at all con-

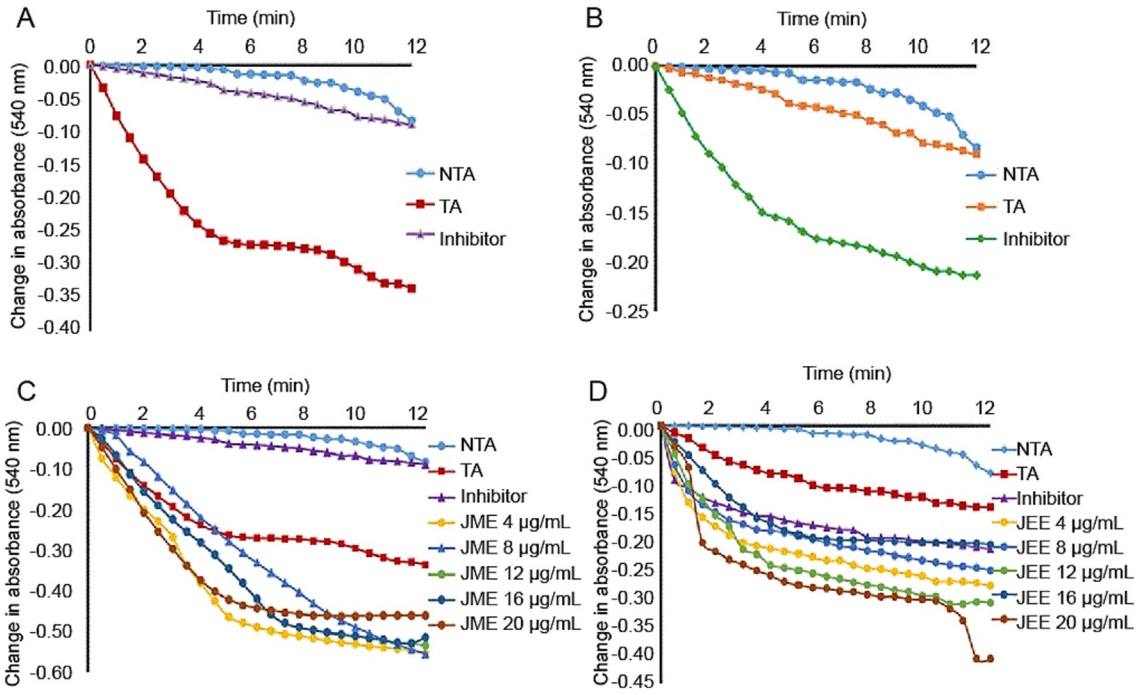

Fig. 2. *In vitro* induction of mitochondrial membrane permeability transition pore by calcium (a standard inducer of the pore) by pre-incubation (A). *In vitro* induction of mitochondrial membrane permeability transition pore by calcium (a standard inducer of the pore) by incubation with calcium (B). *In vitro* induction of rat liver MMPT pore by varying concentrations of JME (pre incubation with extract) (C). *In vitro* induction of normal rat liver MMPT pore by varying concentrations of JEE (pre-incubation with extract) (D). NTA: without triggering agent. TA: swelling with Ca^{2+} as the triggering agent. Inhibitor: spermine inhibition.

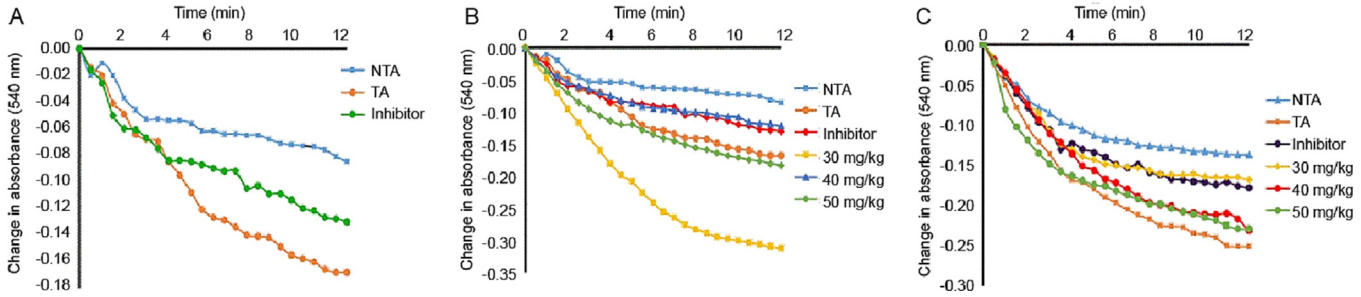

Fig. 3. Changes in absorbance (540 nm) over 12 min of assessing mitochondrial permeability transition in rat liver mitochondria (control) in the presence of calcium and concomitant reversal with spermine (A). Comparison of the *in vivo* inductive effect of varying doses (30, 40, 50 mg/kg) of JME in comparison with control (B). Comparison of the *in vivo* inductive effect of varying doses (30, 40, 50 mg/kg) of JEE in comparison with control (C). NTA: without triggering agent. TA: triggering agent. Inhibitor: spermine inhibition.

centrations. When compared to the control, there was a robust and distinct significant increase at concentrations of 12, 16, and 20 µg/mL, with the highest increase of mATPase activity found at 16 µg/mL. JEE-treated rats exhibited significant activity at 8, 16 and 20 µg/mL, with a non-significant increase in activity at 4 µg/mL. This confirmed our prior findings that JME was more effective at opening the MMPT pore (Fig. 3). The activity of ATPase increased significantly at 30 and 40 mg/kg in the JME treated group while a significant increase was observed at all concentrations in the groups treated with JEE (Fig. 4B). Hence, ATP hydrolysis was enhanced by both extracts.

3.4. In vitro and in vivo mitochondrial lipid peroxidation

The ability of JME and JEE to generate lipid peroxides (MDA) *in vitro* was tested and the result was displayed in Fig. 5A. At the various concentrations tested with JME, MDA was generated. At 4 and 8 µg/mL, MDA were increased and highly significant ($P < 0.001$) followed by 12, 16, and 20 µg/mL. JEE also increased the level of MDA significantly ($P < 0.001$). Fig. 5B showed *in vivo* mitochondrial lipid peroxidation being quantified with levels of MDA released. The result showed that in the JME-treated rats, the level of MDA was increased significantly ($P < 0.001$) at all concentrations with the highest being at 30 mg/kg. In the JEE-treated rats, MDA released was highly significant and remarkable at both 30 and 40 mg/kg when compared with the control. The highest level of MDA was observed to be at 40 mg/kg and the lowest at 50 mg/kg.

3.5. Assay of liver enzyme activity

Serum ALT and AST are biomarkers of liver damage. JME-treated rats were found to increase the levels of AST at 30 and 40 mg/kg significantly ($P < 0.01$) but at 50 mg/kg, a non-significant difference was observed when compared to the control. The increase in ALT level was not significant in comparison to control. The level of ALP was however decreased at all concentrations while the GGT level was close to the control and significantly ($P < 0.05$) reduced at 50 mg/kg (Fig. 6A). AST level was decreased at 30 and 40 mg/kg in JEE treated rats with a non-significant increase at 50 mg/kg.

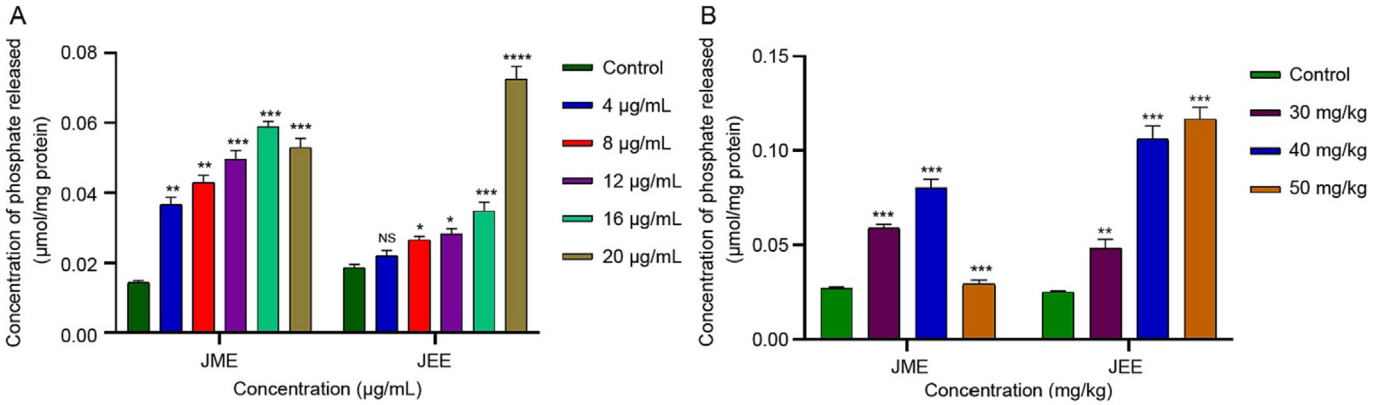

Fig. 4. *In vitro* (A) and *in vivo* (B) induction of normal rat ATP hydrolysis by mitochondrial ATPase activity by varying concentration of JME and JEE (mean ± SEM, n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* control group.

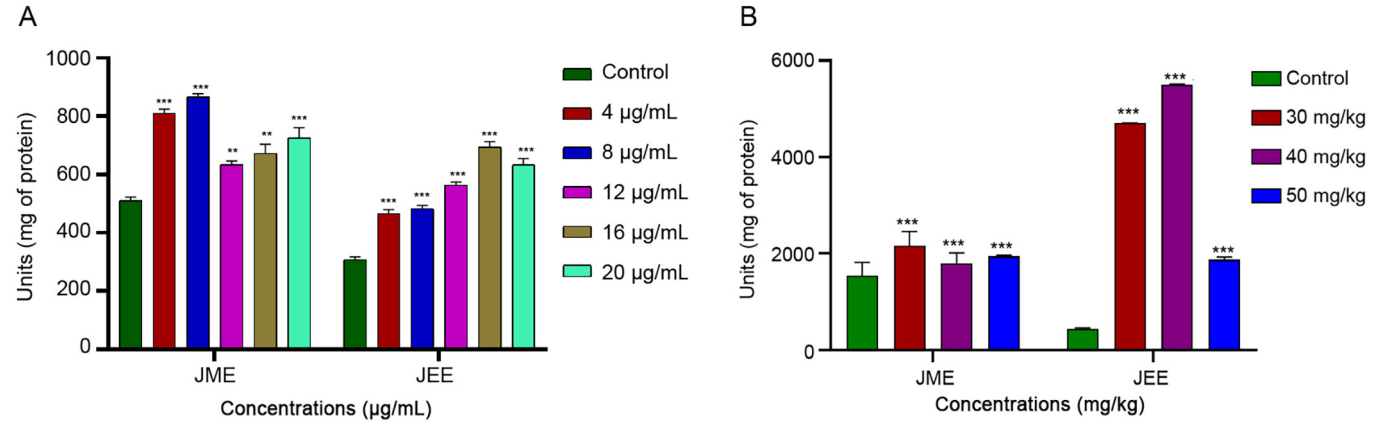

Fig. 5. *In vitro* (A) and *in vivo* (B) mitochondrial lipid peroxidation quantified in terms of MDA levels in control and treatment male Wistar rats by varying concentrations of JME and JEE (mean ± SEM, n = 5) $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* control group.

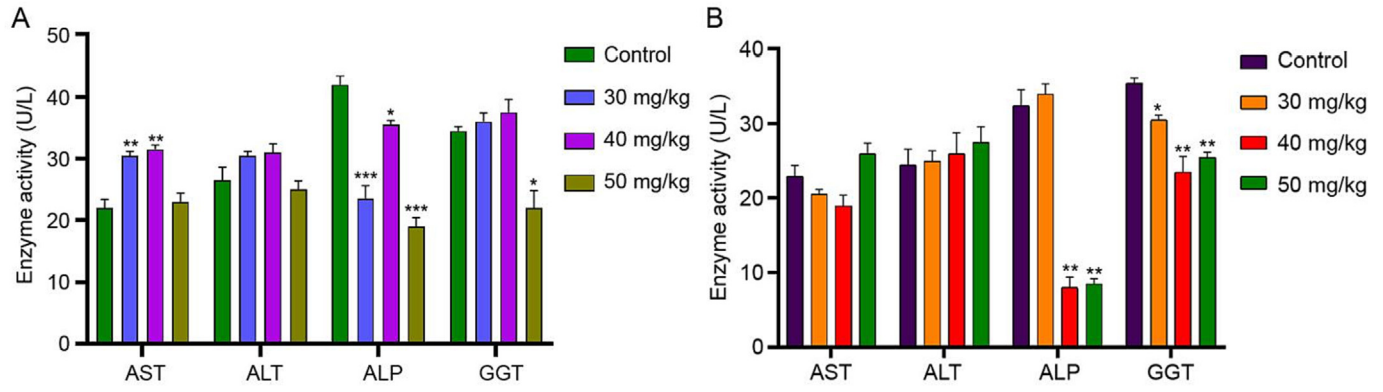

Fig. 6. Effects of JME (A) and JEE (B) on liver enzymes (mean ± SEM, n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* control group. AST: aspartate aminotransferase, ALT: alaline transaminase, ALP: alkaline phosphatase, GGT: gamma glutamyl Transferase.

The level of ALT was slightly increased. ALP level was observed to be significantly reduced at 40 and 50 mg/kg and slightly increased at 30 mg/kg. However, GGT levels were reduced at all concentrations significantly (P < 0.05, 0.01) (Fig. 6B).

4. Discussion

Reports from several studies are that extracts of herbal mixtures are excellent agents for the treatment of various diseases including cardiovascular, antioxidative, and anticancer effects (Aung et al., 2017; Shaito et al., 2020). Toxicity and safety profiling are important considerations when evaluating natural product clinical applications (Li et al., 2021) Acute toxicity testing is used to determine acute oral toxicity and thus the dose that will result in death. Oral administration of JME results in an LD_{50} of 3 808 mg/kg bw, however even a higher dose of extract, 5 000 mg/kg, did not produce toxicity or death in the animals in the current study. As a result, even at 5 000 mg/kg, plant extract may be considered safe.

The histopathology results showed mild toxicity at the higher dose (Fig. 1). When compared to individual plant parts, the methanol extract of the Jatonik polyherbal mixture had a higher LD_{50} (3 808 mg/kg) with *A. conyzoides* having 1 275 mg/kg (Adesanwo et al., 2019), *X. aethiopica* having 3 464 mg/kg (Ayodele et al., 2019), and *H. umbellate* having 1.597 9 g/kg (Osagie & Osemwenkhae, 2011) and this could be as a result of the synergistic effect of the plant parts which lead to decreased toxicity and better therapeutic outcome (Han et al., 2019; Madić et al., 2019) hence the need for carrying out acute toxicity on plant extract mixtures.

Considered attractive targets for the development of new cancer therapies is the targeting of mitochondria for apoptosis-inducing pathways that in various cancers are habitually defective (Boyenle et al., 2022; Galluzzi et al., 2010). Previous work on the plant parts used revealed that *X. aethiopica* fruit extract induced apoptosis (Adaramoye et al., 2011), displayed cytotoxicity against multi-drug resistant (MDR) cancer cell lines (Kuete et al., 2013, 2015) and was also reported to induce MPT pore and Caspase-3 (Choumessi et al., 2012; Ribeiro et al., 2021). *A. conyzoides* was also reported to induce mitochondrial-mediated apoptosis (Olowofolahan & Olorunsogo, 2021).

In vitro MMPT pore opening of the intact mitochondria was observed when pre-incubated before adding calcium, however when incubated with calcium, the degree of MMPT pore opening was reduced (Fig. 2). At varying concentrations of 4, 8, 12, 16 and 20 μg/mL of JME, MMPT pore was induced in a concentration-dependent manner by 569 %, 570 %, 545 %, 520 % and 457% at 4, 8 12, 16 and 20 μg/mL and having the highest induction at the lowest concentration while JEE also induced the pore opening but was decreased when compared with JME with 392%, 273%, 237%, 205%, and 151% at 20, 12, 4, 8 and 16 respectively. The observed opening could be due to mitochondria injury according to Oyebode and co-workers (Oyebode et al., 2021). In contrast to JME, the highest induction was observed at 20 μg/mL for the JEE (Fig. 2D). At varying concentrations (30, 40, 50 mg/100 g bw) of JME and JEE, *in vivo* MMPT pore opening was observed. For the JME, the highest induction was observed at 30 mg/100 g bw (Δ_{540} nm = −0.312) with a 1.5-fold increase and the observed induction was reversed by spermine (Fig. 3B). JEE also induced the MMPT pore with the highest induction observed at 50 mg/100 g BW (Δ_{540} nm = −0.229) (Fig. 3C) suggesting that there are some phytochemicals present in the extract that are capable of altering the integrity of the mitochondria (Kintzios, 2006). When compared to the control, the MDA (Fig. 5) and mitochondrial ATPase activity (Fig. 4) were significantly elevated in the treatment groups (JME and JEE) both *in vitro* and *in vivo*.

The effects of the administration of JME and JEE on the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were assayed respectively as shown in Fig. 6. ALT and AST are enzymes involved in amino acid and carbohydrate metabolism while hydrolysis of phosphate bonds involves ALP (Shittu et al., 2015). Evaluation of these parameters is important to know the integrity of the liver function after they have been exposed to plant extracts or chemical substances (Lawal et al., 2016). JME and JEE significantly decreased the ALP activity compared to the control. JME and JEE non-significantly increased the ALT activity at 30, 40 mg/kg bw which could be a result of low antioxidants while administration of JEE decreased the activity of AST in the liver and JME increased the activity at low concentrations. GGT activity also decreased significantly ($P < 0.05$) at the highest concentration. It can therefore be deduced from this result that the JME and JEE extract shows a lesser risk of tissue damage except at high concentrations.

5. Conclusion

The methanol and ethylacetate extract of the Jatonik polyherbal mixture induced the opening of the MMPT pore. The *in vitro* and *in vivo* data here suggested that the presence of pro apoptotic properties in JEE and JME made it a polyherbal mixture open for novel discoveries and thus be considered for further studies. The results also showed both extracts showed a mild effect on the liver and the level of the enzymes was highly decreased at a higher dosage hence the extracts can be utilized safely at low doses for therapeutic use in pharmaceutical formulations. Bioassay-guided fractionation may give a clue as to which bioactive compound is responsible for these activities, opening up the discovery of nature-friendly therapeutics for pathological conditions like cancer.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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