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Research article

Ethanol leaf extract of *Psychotria microphylla* rich in quercetin restores heavy metal induced redox imbalance in rats

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ABSTRACT

Psychotria microphylla is a plant found in Africa and many parts of the world where the leaves are locally used in folk medicine for the treatment of toxicity related liver diseases. We investigated the antioxidant potentials of ethanol leaf extract of *Psychotria microphylla* (ELE-PM) in restoring hepatic redox dysregulations in rats exposed to heavy metals. HPLC was used in quantifying the bioactive compounds in ELE-PM. DPPH (1,1-diphenyl-2 picrylhydrazyl), FRAP (Ferric reducing antioxidant power) and NO (Nitric Oxide) assays were used for *in vitro* studies. The *in vivo* studies involved 30 rats randomly divided into 5 groups ($n = 6$). Group 1 received normal saline (2 mg/kg), group 2, 3, 4 and 5 received a combined solution of $Pb(NO_3)_2$ (11.25 mg/kg) and $HgCl_2$ (0.4 mg/kg) respectively. After 7 days of heavy metal exposure, groups 3, 4 and 5 received a daily bolus administration of 200, 400 and 600 mg/kg body weight of EE-PM respectively through oral intubation for 28 days. HPLC quantification revealed a high amount of quercetin (27.43 ± 0.04 mg/100g), lower amounts of gallic acid (7.60 ± 0.06 mg/100g) and rutin (0.38 ± 0.009 mg/100g). Additionally, ELE-PM demonstrated strong inhibitory potentials against free radical scavenging activity generated *in vitro*. More interestingly, administration of ELE-PM significantly ameliorated hepatic redox dysregulations elicited by the exposure of the rats to heavy metals in a dose dependent pattern. ELE-PM is highly rich in flavonoid compound quercetin and perhaps this may be responsible for the strong antioxidant potentials exhibited in this investigation.

1. Introduction

Global environmental pollution and contamination with heavy metals have been on the increase since the 18th century industrial revolution. This has continued to be a major health problem across the globe (Siddiqa and Faisal, 2020). More so, with recent enormous advancement in technological development and industrialization, heavy metals are now ubiquitously present in the environment with consequent negative impact on all biological systems both plants and animals (Hussain et al., 2020; Wallace and Djordjevic, 2020; Shen et al., 2020; Hareram et al., 2020). Of particular economic importance amongst these heavy metals are Mercury and Lead. Mercury when bio-accumulated in the body system through various environmental sources produces several disease conditions in the body (Guzzi et al., 2020). It has toxic effects on the reproductive, digestive, neurological and immune systems of the body (Boujbiha et al., 2012; Oliveira et al., 2020a, b; Lu and Khera, 2020; Mergler et al., 2007). Lead causes morphological, physiological and biochemical alterations in various organ systems of the human body (Cassleman et al., 2020; AL-Megrin et al., 2020; Abdelhamid et al., 2020; Abdel Moneim, 2016). Both of these heavy metals are commonly used in various industrial processes and are mostly deposited in the environment leading to contamination and subsequent toxicity to biological life (Levin et al., 2020).

Psychotria microphylla Elmer is an evergreen shrub with a slender stem found in many parts of the world. It belongs to the tribe of *Psychotrieae (Rubiaceae)*, which consist of about 2000 species. It grows in swampy forest and is commonly used for fishing in local communities of Afikpo, South Eastern Nigeria. Species of the genus *Psychotria* L. are used in folk medicine as tea and extracts of other *Psychotria* species have shown anti-inflammatory, antioxidant and analgesic activities respectively (Elisabetsky et al., 1995; Dunstan et al., 1997). Furthermore, crude extract of *Psychotria microphylla* was reported to have nontoxic effect on rats as demonstrated by Orji et al. (2018). It also has strong antibacterial activity (Udu-Ibiam et al., 2015).

Studies have shown that one major mechanism of heavy metal toxicity in biological systems is through induction of redox imbalance in

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the physiological milieu and particularly in various organ systems of the body (Elblehi et al., 2019; Cariccio et al., 2019). Additionally, the use of plant extracts in treatment of oxidative stress due to heavy metal toxicity is an emerging area of research in pharmacology and toxicology (Ivana et al., 2020). However, further empirical investigations aimed at generating sufficient scientific evidence, which would enhance the pharmaceutical/therapeutic potentials of these plant extracts have been advocated (Stagos, 2020; Diederich, 2020). We therefore investigated in this present study if ethanol extract of *Psychotria microphylla* could have the antioxidant potentials to be able to normalize the hepatic redox imbalance elicited due to exposure of rats to heavy metals.

2. Materials and methods

2.1. Collection of sample, identification and preparation of extract

Fresh leaves of *Psychotria microphylla* ("*Oye*" in Igbo, South Eastern Nigeria) were collected from Afikpo, South Eastern Nigeria at the beginning of spring. It was taxonomically identified by Prof Onyekwelu of the Department of Applied Biology Ebonyi State University, Nigeria. A sample of the plant was deposited at the herbarium of the Department with the voucher number of EBSU/APB/HB/0282. The leaves were thoroughly washed under clean running water and dried under a shade for 24 h. It was further pulverized into powder with laboratory blender and 300 g of the ground leaves was soaked in 1 L of ethanol for two days with intermittent shaking using water bath shaker. The mixture was further sieved using a clean muslin cloth and the ethanol was recovered under mild temperature of 30 °C to get the extract that was used for the study.

2.2. Chemicals and reagents

Pure flavonoid compounds (quercetin and rutin hydrate) and phenolic acid compounds (gallic, ferrulic and caffeic acids) used as standards in HPLC studies were purchased from sigma-Aldrich (UK). Methanol, acetonitrile, Phosphoric acid, Lead Nitrate, Mercury Chloride, Ethanol and Water (HPLC grade) were purchased from Thermo Fisher Scientific (UK). Other reagents used for various assays were all of standard analytical grade.

2.3. HPLC apparatus and quantification of bioactive compounds (flavonoids and phenols) in ELE-PM

The leaf extract was quantitatively evaluated to determine the amount of flavonoids and phenolic acid compounds present in the extract using Agilent 1100 Series LC HPLC equipped with column thermostat, UV detector and a 20 μL injection loop (Agilent Technologies, CA, USA). Furthermore, the chromatographic separation process was achieved using a ZORBAX SB-C18 analytical column (250 mm × 4.6 mm, 5 μm) at wavelength of 257 nm. The mobile phase was made up of 0.01 mol.L–1 phosphoric acid-methanol-acetonitrile (72:14:14, v/v/v). The protocol followed was as described by Akomolafe et al. (2015) and Agilent catalogue 2017.

2.4. In vitro antioxidant studies

2.4.1. DPPH assay for evaluation of free radical scavenging activity

The free radical scavenging activity of ELE-PM was assayed using DPPH (1,1-diphenyl-2 picrylhydrazyl) reagent (Sigma Aldrich USA) as described by Gyamfi et al. (1999). The concentrations of the extract used in this assay were 25, 50, 75 and 100 μg/ml respectively.

2.4.2. Nitric oxide radical scavenging activity assay

The nitric oxide radical scavenging potentials of ELE-PM was determined using the method described by Igbinosa et al. (2011). The concentrations of the extract used were 25, 50, 75 and 100 μg/ml respectively.

2.4.3. Ferric reducing antioxidant power (FRAP) assay

The reducing power of ELE-PM was evaluated according to the method described by Pulido et al. (2000). The concentrations of the extract used in this assay were 25, 50, 75 and 100 μg/ml respectively.

2.5. In vivo antioxidant studies

2.5.1. Experimental design and animal handling

The International Standard Procedure for use and care of Experimental Animals as found in the US guidelines of National Institute of Health (NIH publication #85–23, revised in 1985) which has been adopted by Department of Biochemistry, Ebonyi State University Ethical Committee Board was followed throughout the experiment. The study was approved by the Departmental Institutional Ethical Committee of Biochemistry Department, Ebonyi State University Abakaliki, Nigeria. The Ethical approval number is EBSU/BCH/ET/18/011. Thirty albino rats with body weight ranging from 180-200 g were purchased from University of Nigeria Nsukka Enugu, Nigeria. There were kept and acclimatized within the standard animal house of Biochemistry Department with free access to feeds (commercial rat chow) and clean water. They were randomly distributed into 5 groups (n = 6). Group 1 received normal saline (2 mg/kg), group 2, 3, 4 and 5 received a combined solution of $Pb(NO_3)_2$ (11.25 mg/kg) and $HgCl_2$ (0.4 mg/kg) respectively. After 7 days of heavy metal exposure, groups 3, 4 and 5 received a daily bolus administration of 200, 400 and 600 mg/kg body weight of EE-PM respectively through oral intubation for 28 days. The choice of the doses were based on the toxicological studies of Orji et al. (2018). The choice of heavy metal doses exposed to the rats was according to studies of Goudarzi et al. (2017). At the end of the investigations, the animals were allowed a one-day rest and then euthanized. The liver was excised, rinsed with normal saline and homogenized in ice-cold buffer and used for various oxidative stress analyses.

2.5.2. Determination of MDA level

Malondialdehyde (MDA) levels in the liver homogenates of the rats was determined through spectrophotometry by using the method of Buege and Aust (1978).

2.5.3. Determination of the activities of antioxidant enzymes (SOD and CAT)

Superoxide Dismutase (SOD) activity in the liver homogenates of the rats was determined according to the method described by Sun and Zigma (1978) while catalase (CAT) activity was determined according to the method of Oyedemi et al. (2010).

2.5.4. Determination of reduced glutathione (GSH) level

Reduced glutathione (GSH) level in the liver homogenates of the rats was evaluated according to method described by Aguirre et al. (2011).

2.5.5. Determination of liver function markers

Alkaline phosphatase was assayed according to the method of Wright et al. (1972). Bilirubin was determined using the method of Jendrassik and Grof (1938) while the method described by Reitman and Frankel (1957) was used to assay for the activities of alanine aminotransferase and aspartate aminotransferase respectively.

2.6. Statistical analysis

The statistical analysis were done using Graph Pad Prism 5.04 (GraphPad, La Jolla, CA, USA). Data was expressed as Mean ± SEM. One-way ANOVA with Dunnett posthoc test was further used for the statistical tests. Generally, $p < 0.05$ was considered as the statistical level of

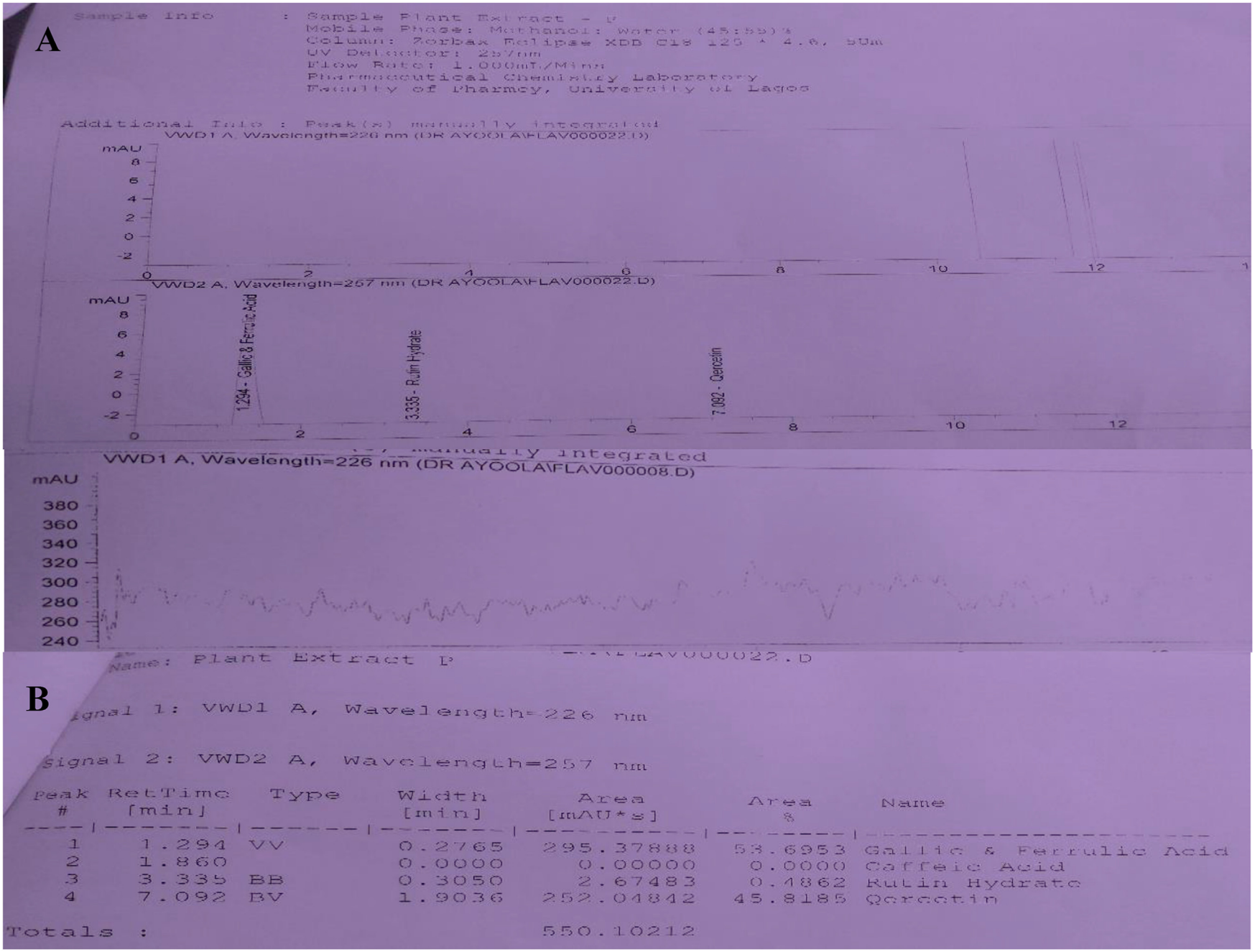

Figure 1. Figure 1 shows the HPLC Chromatogram of Ethanol Leaf Extract of *Psychotria microphylla* (A) Chromatogram graph showing the peak (B) Chromatogram data values. The result revealed the presence of flavonoid compounds (quercetin and rutin) and phenolic compounds (gallic acid and ferulic acid). Caffeic acid was not detected in the extract. The area under the peak for quercetin, gallic acid and ferulic acids respectively were high, which is an indication of high concentration of these compounds in the leaf extract. The concentrations in mg/100g are shown in Table 1.

Table 1. HPLC Quantitative Analysis of Bioactive compounds present in ELE-PM.

Compound	Amount (mg/100g)
Gallic acid & Ferulic acid	7.60 ± 0.06
Quercetin	27.43 ± 0.04
Rutin	0.38 ± 0.009
Caffeic acid	*ND

* Not Detected. Data are presented as Mean ± SD of triplicates.

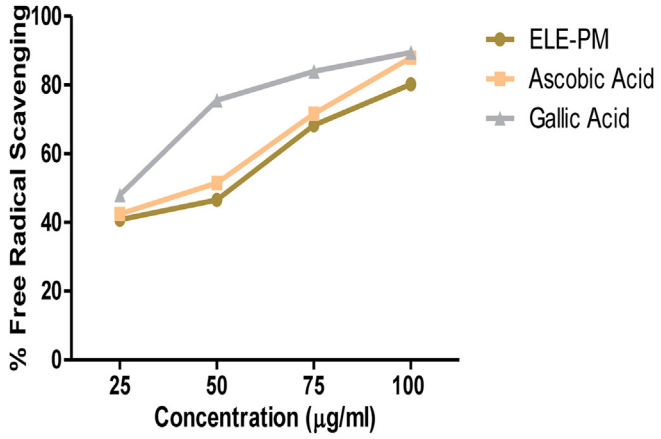

Figure 2. Free Radical Scavenging Potential of ELE-PM using DPPH Assay.

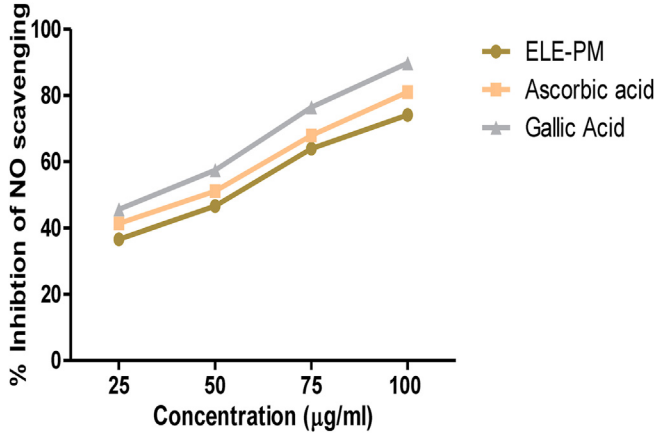

Figure 3. Nitric Oxide Free Radical Scavenging Activity of ELE-PM using NO Assay.

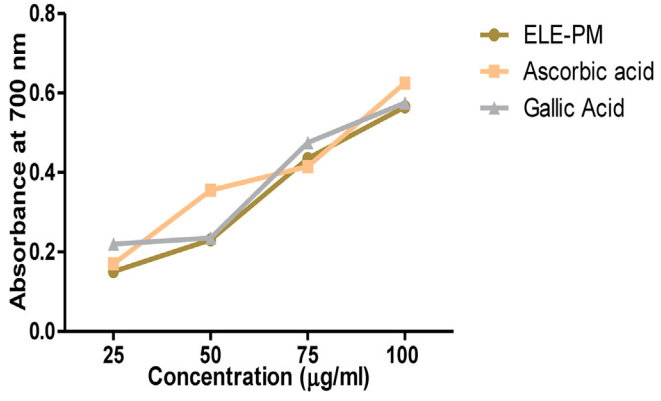

Figure 4. Total Reducing Power of ELE-PM using FRAP Assay.

significance. The IC_{50} for DPPH and NO assays respectively were calculated using Linear Regression.

3. Results

Table 1.

3.1. In vivo antioxidant studies

3.1.1. DPPH Free radical scavenging activity of ELE-PM

Figure 2 shows the free radical scavenging potential of ELE-PM through DPPH assay. The result revealed a concentration dependent increase in the free radical scavenging activity of ELE-PM, which strongly compares with the pure compounds used as standards particularly ascorbic acid. The IC_{50} of ELE-PM is 46.50 ± 0.8300 µg/ml while ascorbic acid is 41.13 ± 0.6280 µg/ml and gallic acid is 16.88 ± 0.1474 µg/ml .

3.1.2. Nitric oxide free radical scavenging activity of ELE-PM

Figure 3 shows the Nitric oxide free radical scavenging activity of ELE-PM. The result revealed a near parallel pattern of scavenging activity for ELE-PM and the compounds. The scavenging activity increased in direct concentration dependent pattern and highly comparable to each other. The IC_{50} for ELE-PM is 52.22 ± 0.4092 µg/ml while ascorbic acid is 43.43 ± 0.03759 and gallic acid is 33.85 ± 0.03734.

3.1.3. FRAP total reducing power of ELE-PM

Figure 4 shows the total reducing power of ELE-PM using FRAP assay. The result revealed a concentration dependent increase in the reducing power of ELE-PM, which also compares strongly with the pure compounds used as standards. At 100 µg/ml concentration, the reducing power measured in absorbance for the compounds were not far apart. The absorbance for ELE-PM was 74.12, while ascorbic acid was 81.055 and gallic acid was 89.785.

3.2. In vivo studies

3.2.1. Effect of ELE-PM on oxidative stress markers in rats exposed to heavy metals (Pb and Hg) toxicity

Figure 5 (a-b) shows the levels of reduced glutathione (GSH) and Malondialdehyde (MDA) respectively in the liver homogenate of the rats. The result revealed that exposure of the rats to the combined solution of the heavy metals (Pb and Hg) led to a significant decrease in the level of GSH in the liver whereas MDA level was significantly elevated in the rats due to exposure to the heavy metals. Interestingly, daily bolus administration of ELE-PM normalized this reduced level of GSH and elevated level of MDA in a dose dependent pattern respectively with 600 mg/kg dose completely normalizing their levels to same levels with the normal rats.

3.2.2. Effect of ELE-PM on antioxidant enzymes in rats exposed to Heavy Metals (Pb and Hg) Toxicity

Figure 6 (a-b) shows the Catalase (CAT) and Superoxide Dismutase (SOD) activities in the liver homogenates of the rats exposed to heavy metals toxicity respectively. The result revealed a significant decrease in the activities of the enzymes on exposure of the rats to the heavy metals. Moreover, administration of ELE-PM reversed this reduction in activity in a dose dependent pattern. As with other antioxidant assays in this investigation, 600 mg/kg concentration of the extract completely normalized the activities of CAT and SOD to levels comparable to the normal rats.

3.2.3. Effect of ELE-PM on liver function markers in rats exposed to Heavy Metals (Pb and Hg) Toxicity

Figure 7 (a-f) shows the activities of liver enzymes (ALT, AST and ALP) and the levels of other liver function makers (albumin, total

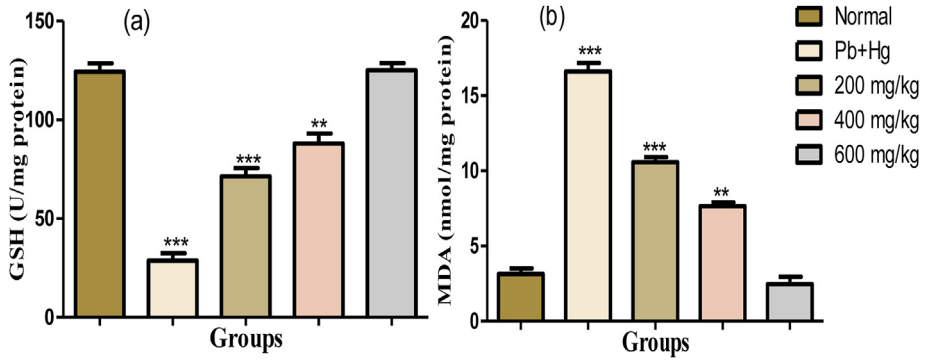

Figure 5. (a–b). Effect of ELE-PM on Markers of Oxidative Stress in Rats Exposed to Heavy Metals (Pb and Hg) Toxicity. (a) GSH and (b) MDA levels in the liver homogenate of the rats. All the groups were compared to the normal control using Dunnett posthoc test and ranked according to their levels of significance using asterisk. Group without asterisk shows no significant difference when compared to the normal control.

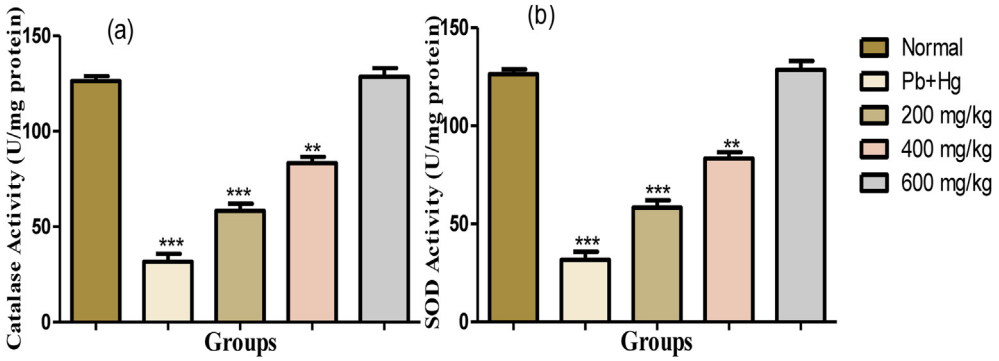

Figure 6. (a–b). Effect of ELE-PM on Antioxidant Enzymes in Rats Exposed to Heavy Metals (Pb and Hg) Toxicity. (a) Catalase (CAT) and (b) Superoxide dismutase (SOD) Activities in the Liver Homogenates of the Rats. All the groups were compared to the normal control using Dunnett posthoc test, and ranked according to their levels of significance using asterisk. Group without asterisk shows no significant difference when compared to the normal control.

bilirubin and total protein) in the serum of rats exposed to heavy metals. The result revealed that exposure to heavy metals caused significant elevation in the serum activities of the liver enzymes investigated. Heavy metals exposure also caused a significant increase in the levels of total bilirubin but decreased the serum levels of albumin and total proteins respectively. Interestingly, a daily bolus administration of ELE-PM especially 600 mg/kg ameliorated the deleterious effects of the heavy metals on the liver by reversing the dysregulations of these important liver function biomarkers.

4. Discussion

Based on the ethnopharmacological relevance and folk medicinal uses of *Psychotria microphylla* in the treatment of liver diseases amongst the locals in Eastern Nigeria, we investigated the antioxidant potentials of its ethanol leaf extract in ameliorating hepatic redox imbalance induced by exposure of rats to heavy metal compounds. Firstly, we used HPLC to screen and quantify the bioactive compounds present in the leaf extract. Interestingly, our result revealed the presence of flavonoids and phenolic acid compounds in the extract at varied concentrations. Quercetin, a well-known flavonoid (Batiha et al., 2020; Huang et al., 2020) was highest followed by gallic acid and ferulic acid, which are phenolic acid compounds respectively. Rutin, a flavonoid compound was in trace amount while caffeic acid was not detected. Quercetin is a much studied flavonoid compound with various bioactive properties. Studies have shown that it possesses antioxidant, anticarcinogenic, antiviral, antithrombotic and anti-inflammatory potentials respectively (Moosavi et al., 2016; Kanimozhi et al., 2017). Gallic acid also possesses strong antioxidant potential (Khan et al., 2020; Ezzati et al., 2020; Abarikwu et al., 2017), while the antioxidant potential of rutin has also been investigated by others (Manzoni et al., 2020; Liu et al., 2020; Sharma et al., 2020; Halvorsen et al., 2006). Thus, the presence of these bioactive compounds in ELE-PM suggests that leaves of *Psychotria microphylla* could be useful as raw materials in pharmaceutical preparations of antioxidants.

Furthermore, to verify if these bioactive compounds present in ELE-PM could exert real-time antioxidant potentials, we further conducted *in vitro* antioxidant studies using well-known antioxidant assays (NO, FRAP and DPPH). Nitric oxide is one of the major oxygen free radicals that has been implicated in the distortion of normal redox system of the body. Additionally, it has been associated to the pathology of various disease conditions in the body such as cancer, cardiovascular diseases and the natural body aging process (Radi, 2018; Amirtharaj et al., 2017). Our NO *in vitro* assay result revealed that ELE-PM strongly inhibited the free radical scavenging activity of NO in a dose dependent pattern comparable to standards used. Interestingly, the ELE-PM has a low IC_{50} of 52.22 ± 0.4092 μg/ml, which implies that at this concentration, 50% of the NO free radicals were inhibited by ELE-PM. This is comparable to the IC_{50} of ascorbic acid (43.43 ± 0.03759) and gallic acid (33.85 ± 0.03734), which were used as standards in the study. This inhibitory activity is slightly comparable to that of the plant extracts reported by Adebayo et al. (2019) and lower than the value reported by Suluvoy and Berlin Grace (2017) for *Averrhoa bilimbi* L. fruit extract. FRAP assay measures the ability of any compound to convert Fe^{3+} to its reduced state Fe^{3+}, which is an indicator of total antioxidant capacity of such compound (Haida and Hakiman, 2019). We reported a concentration dependent rapid increase in the total reducing power of ELE-PM, which strongly correlated with that of the standards used. Studies have shown that phenolic and flavonoid compounds have strong ferric reducing power (Eraslan et al., 2007; Sethi et al., 2020). This is consistent with our HPLC findings in this present study. More so, DPPH assay is a key *in vitro*

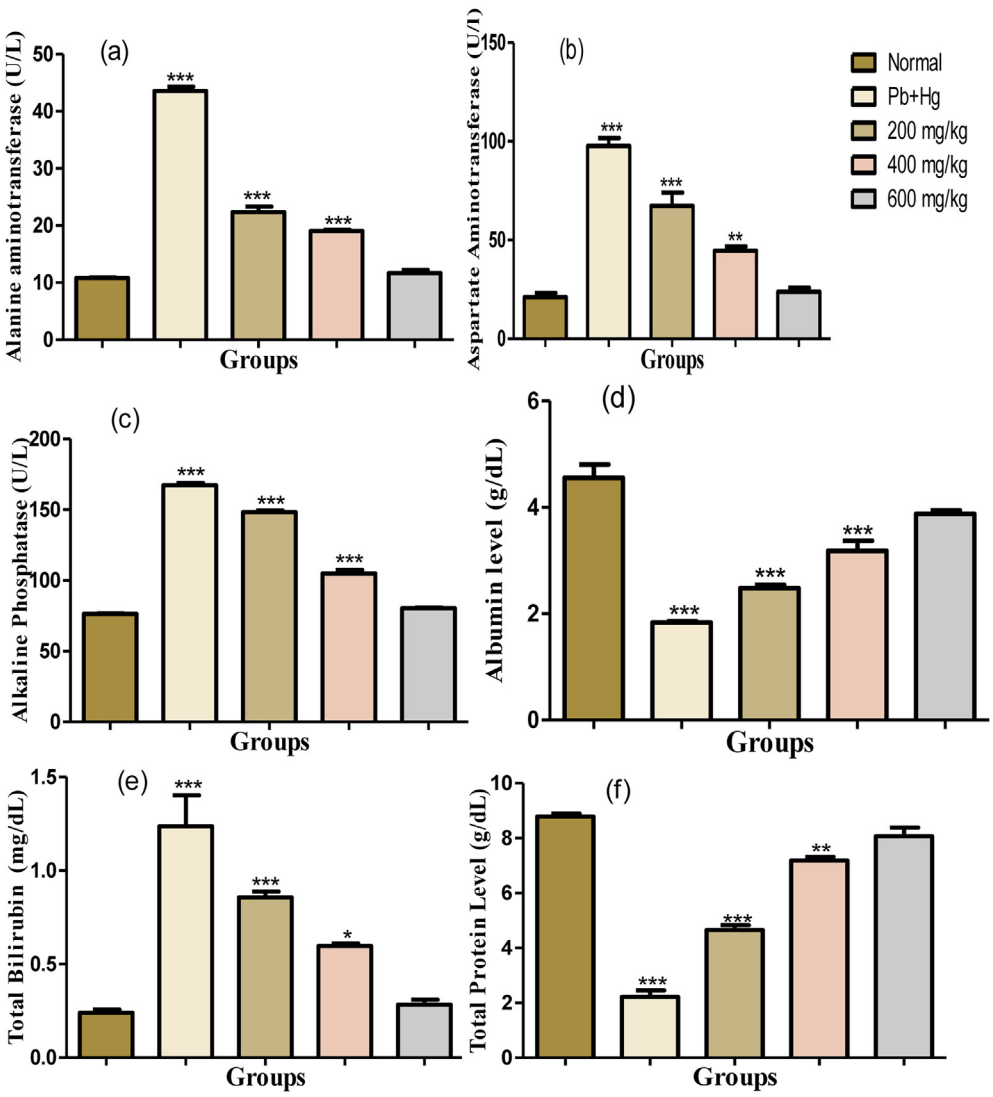

Figure 7. (a-f). Effect of ELE-PM on Liver Function Markers in Rats Exposed to Heavy Metals (Pb and Hg) Toxicity. (a) Alanine aminotransferase (ALT), (b) Aspartate aminotransferase (ASP), (c) Alkaline Phosphatase (ALP), (d) Albumin, (e) Total Bilirubin, (f) Total Protein. All the groups were compared to the normal control using Dunnett posthoc test, and ranked according to their levels of significance using asterisk. Group without asterisk shows no significant difference when compared to the normal control.

assay in ascertaining the antioxidant potentials of compounds. Our result revealed a consistent concentration dependent increase in the percentage inhibition of the free radicals produced in the assay, which suggests that ELE-PM has strong antioxidant potential. The IC_{50} of ELE-PM is 46.50 ± 0.8300 μg/ml; this is lower than the values obtained by Samardžić et al. (2018) and do Nascimento et al. (2018) for some of the samples tested. This also compares strongly with that of ascorbic acid, 41.13 ± 0.6280 μg/ml, which was one of the standards used in the assay.

Moreover, to ascertain whether the observed *in vitro* antioxidant effect of ELE-PM could be obtainable *in vivo*, we exposed rats to heavy metals and investigated several well-known *in vivo* oxidative stress biomarkers (GSH, MDA, SOD and CAT) on the liver homogenates of the rats respectively. We also investigated liver function biomarkers (ALT, ASP, ALP, Albumin, Total bilirubin and Total protein) to biochemically ascertain the level of damage in the liver due to the accumulation of the heavy metals. Studies have shown that one key mechanism of toxicity of heavy metals in the physiological and organ systems of the body is through distortion in the redox system of the body (Durak et al., 2010; Goudarzi et al., 2018). This usually begins with abnormal increase in generation of ROS in the body due to detoxification process of heavy metals in the kupffers cells of the liver leading to redox imbalance and oxidative stress. Subsequently, the increased oxidative stress aggravates remarkably and forms the basic pathophysiology of several diseases such as cancer, cardiovascular diseases, diabetes and neurodegenerative disorders in the body (Luo et al., 2020; Ren et al., 2020; Prasad and Srivastava, 2020; Nabavi et al., 2013). We confirmed this established knowledge across all the oxidative stress and liver function biomarkers investigated in this present study. Reduced glutathione (GSH) is a major molecular non-enzymatic antioxidant found in various cells and tissues of the body where they perform important functions in the overall redox system of the body. Dysregulation of GSH level is critical in the development of various diseases in the body (Adeoye et al., 2018; Kart et al., 2016). Our result revealed that exposure of the rats to heavy metals caused a significant reduction in the level of GSH in the liver of the rats, which perhaps may have altered the redox system of the hepatocytes. The heavy metals may have bound to the sulfhydryl group of GSH, which further inhibited its antioxidant functions. However, administration of various doses of ELE-PM caused a dose dependent significant increase in GSH level, which is a positive trend consistent with the *in vitro* studies. Bioactive components in ELE-PM may have increased the synthesis of GSH and/or enhanced the detoxification actions of the kupffer cells of the liver through scavenging of free radicals produced during the detoxification process of the heavy metals by kupffer cells of the liver.

Additionally, lipid peroxidation is a well-known mechanism of cellular damage in the biological system. MDA is an extremely reactive 3-carbon dialdehyde and the main oxidative product of unsaturated fatty acids in the membranes with toxic property. It is a common biomarker of membrane lipid peroxidation, resulting from the interaction between

ROS and cellular membrane. It is also implicated in various human diseases (Joshi et al., 2017; Leena et al., 2011). Markedly, we reported a significant elevation in the level of MDA in the liver homogenate of rats exposed to the heavy metals. This indicates an increase in ROS generation due to exposure to heavy metals. Heavy metals are known to induce ROS production in the body (Fu and Xi, 2020). Perhaps, the elevation in lipid peroxidation due to ROS induction by the heavy metals may have negatively affected the structural integrity of the liver membrane leading to the leakage of the liver enzymes predominantly localized in the hepatocytes. Additionally, other functions of the liver such as synthesis of albumin and total protein may have been affected as well due to heavy metal accumulation. Our liver function test revealed a significant elevation in the activities of the liver enzymes (ALT, ASP and ALP) in the serum indicating excessive leakage due to possible damage to the cellular membrane of the hepatocytes. Serum levels of total protein and albumin were reduced while total bilirubin was elevated. Serum levels of these biomarkers are markedly used as indicators of severe liver damage (Mishima et al., 2019; Chen et al., 2017). Conversely, daily bolus administration of ELE-PM significantly reversed this abnormal MDA increase especially at 600 mg/kg dosage. Dysregulated liver function biomarkers were also normalized by the administration of ELE-PM especially the most effective dose of 600 mg/kg. Studies have shown that quercetin and gallic acid have the potentials to normalize dysregulated liver function markers and reduce MDA level *in vivo* in various experimental disease conditions (Moosavi et al., 2016; Goudarzi et al., 2017; Kanimozhi et al., 2017). Perhaps, these bioactive compounds earlier identified in ELE-PM through the HPLC studies may be responsible for this striking antioxidant potential in addition to the healing effect on the cellular membrane of the hepatocytes. We further investigated whether there are corresponding effects of both exposure to heavy metals and administration of ELE-PM on the enzymatic antioxidants (catalase and superoxide dismutase). Catalase and superoxide dismutase are important antioxidant enzymes whose activities are interrelated and strongly correlated to the overall regulation of the redox system of the body (Ighodaro and Akinloye, 2018; Leena et al., 2011). Catalase is responsible for breaking down of hydrogen peroxides produced endogenously through normal body metabolism in addition to invasion of foreign substances (Tehrani and Moosavi-Movahedi, 2018). Superoxide dismutase acts on superoxide anion free radical ($O_2^{\cdot}$) and converts it into molecular oxygen and hydrogen peroxide (Younus, 2018). Our result revealed that hepatic catalase and superoxide dismutase activities were decreased on exposure of the rats to the combined solution of the heavy metals. This is consistent with the findings of others (Zhao et al., 2020; Oliveira et al., 2020a, b). Lead and mercury may have decreased the activity of SOD by directly interacting with the –SH group of the enzyme leading to denaturation of the enzyme and subsequent decrease in activity observed in our study Al-Attar (2020); Zhao et al. (2020); Kalantari et al. (2007). Lead is highly deleterious and induces oxidative stress through dysregulation of SOD and catalase (Kumar et al., 2020; Fu and Xi, 2020; Farmand et al., 2005). Additionally, accumulation of these heavy metals in the hepatocyte may have led to their interaction with the amino-acids residue of these enzymatic antioxidants thereby inhibiting their activities. More interestingly, ELE-PM followed the same trend with other oxidative biomarkers investigated in this study and significantly increased the hepatic activities of these important antioxidant enzymes in a dose dependent pattern.

5. Conclusion

The result of our investigation has revealed the enormous unexploited antioxidant potentials of ELE-PM. We have reported for the first time that ELE-PM has bioactive compounds, which may be responsible for the strong antioxidant potentials it demonstrated both in the *in vitro* and *in vivo* studies. This further provides scientific evidence of its local use in folk medicine in South Eastern Nigeria and many other parts of the world in the treatment of liver diseases associated to oxidative stress. Further studies would utilize this baseline data in exploring the potentials of this plant extract.

Declarations

Author contribution statement

J.N. Awoke: Analyzed and interpreted the data; Wrote the paper.
O.U. Orji: Conceived and designed the experiments.
C. Harbor and O. Obasi: Performed the experiments.
I. Igwneyi, N. Ezeani and C. Aloke: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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