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Research Article

Investigation into the Potential of *Paullinia pinnata* (Linn.) Methanol Leaf Extract against Toxicity by Ethylene Glycol Monomethyl Ether in Wistar Rats

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ABSTRACT

Ethylene glycol monomethyl ether (EGME) is a toxicant with wide industrial and domestic use. *Paullinia pinnata* (PP) is a herb with medicinal properties as demonstrated scientifically. The aim of this study is to investigate the possible chemopreventive effect of PP on EGME-induced damagesin the liver and kidney. Seventy adults male Wistar rats were weight-matched into seven groups (n=10). Groups I and II served as controls and received distilled water and 10% dimethyl sulfoxide, respectively. Group III received EGME (200 mg/kg) only. Groups IV-VII each received EGME (200 mg/kg) and PP at 25, 50, 75 and 100 mg/kg doses, respectively. All administrations were done orally daily for 14 consecutive days. On day 15, the animals were euthanized and the liver and kidneys were excised. The EGME significantly ($p<0.05$) reduced the activities and the levels of the enzymatic and non-enzymatic antioxidants. These deleterious effects were prevented by co-administration with PP at 50, 75 and 100 mg/kg dose but not at the 25 mg/kg dose. *Paullinia pinnata* methanol leaf extract barred the toxic effect of EGME at moderate doses in the liver and kidney.

Keywords: *Paullinia pinnata, Ethylene glycol monomethyl ether, Antioxidants*

INTRODUCTION

Ethylene glycol monomethyl ether (EGME), also known as methyl oxitol, monomethyl ethylene glycol ether, methyl glycol, monomethyl ether, monomethyl glycol, 2-methoxy ethanol and commercially as methylcellulose has wide domestic and industrial applications as a result of the hydrophilic and lipophilic properties (Adeyemo-Salami and Farombi, 2018). EGME is used in silk-screen printing, lacquers, paints, photographic and photo lithographic processes, stains, textile and leather finishing, cellulose acetate, inks, cosmetics, surface coating, semi-conductor industry and in the production of food-contact plastics. It is also used as an agent to prevent the freezing of jet fuel and hydraulic fluids (Loh et al., 2008; Takei et al., 2010; Adeyemo-Salami, 2021). However, it is known to be toxic to various tissues and organs in the system including blood, testes, epididymes, liver and kidney (Takei et al., 2010; Bendjeddou and Khelili, 2014; Adeyemo-Salami and Farombi, 2018).

The plant parts of *Paullinia pinnata* (PP) (commonly known as sweet gum) are used for medicinal purposes such as the treatment of fever debility, infectious diseases, post-partum pain, eye ailments, localized pain, whooping cough, diarrhea, rickets and infertility (Burkill, 2000). Some of these have been established scientifically (Adeyemo-Salami

and Ewuola, 2015; Roger et al., 2015; Patience et al., 2017; Adeyemo-Salami et al., 2020).

Globally, plant parts, in the form of herbal medicines or home remedies are being used to treat various ailments (Ekor, 2013). This was corroborated by Pan et al. (2014) who stated that according to the World Health Organization, 75% of the world's populations are using herbs for basic healthcare needs and this includes industrial employees (Oreagba et al., 2011; Kanjanahattakij et al., 2019). This was further reflected in a current publication by World Health Organization (W.H.O., 2019). Therefore, the ingestion of herbal remedies or its active principles, of which P. pinnata is an example, may be able to prevent the toxic manifestations of EGME in industrial employees exposed to it. This would serve as a lead to isolation of the active principle(s) which would be formulated into drugs or food supplements.

Therefore, this study is designed to investigate the possible chemopreventive effect of *P. pinnata* methanol leaf extract on the toxic effect of EGME in the liver and kidney of Wistar rats.

MATERIALS AND METHODS

Study design and location

Ethylene glycol monomethyl ether was a product of LobaChemie (Mumbai, India). All other reagents were either SureChem (U.K.) or Sigma Aldrich (St. Louis, MO, U.S.A.) products.

Collection and preparation of plant sample

The source and authentication of the leaves of *Paullinia pinnata* was at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The leaves were collected during the rainy season in July. It was given the specimen voucher number FHI 106555. The leaves were air-dried, milled and extracted using absolute methanol for 6 hours in a Soxhlet extractor over a steam bath and the extract obtained was 14% of the plant sample.

Experimental animals and care

Ethical approval for the study was granted by the Animal Care and Use Research Ethics Committee of the University of Ibadan, Nigeria and the number UI-ACUREC/ APP/ 10/2016 /003 was assigned. Seventy (70) adult male Wistar rats weighing 140- 190g were obtained from the Department of Veterinary Anatomy, University of Ibadan, Oyo State, Nigeria, and were weight-matched into seven groups of ten animals each. They were taken care of in standard laboratory cages and given feed (Breedwell Feed, Nigeria) and tap water *ad libitum* in the Animal house of the Department of Biochemistry of the same University where they were acclimatized for a week. The 12-hour light/dark cycle was maintained.

Experimental design

Groups I and II served as controls and received distilled water and 10% dimethyl sulfoxide (DMSO; vehicle for PP), respectively. Groups III received EGME (200 mg/kg) constituted with distilled water. Groups IV-VII each received EGME (200 mg/kg) and PP at 25, 50, 75 and 100 mg/kg doses, respectively. For the co-administered groups, EGME was administered first and then PP extract was administered at the various doses an hour after. All treatments were done by oral gavage daily for 14 consecutive days. On day 15, the animals were euthanized by cervical dislocation and the liver and kidneys were excised and weighed. The liver and kidney were then homogenized in Tris-HCl/KCl buffer and the supernatant was stored at -20^{0}C until time for biochemical analyses which were antioxidant assays. The antioxidant assays conducted on the liver and kidney supernatants were superoxide dismutase (SOD) (Misra and Fridovich, 1972); catalase (CAT) (Claiborne, 1985); glutathione-S-transferase (GST) (Habig *et al.*, 1974); glutathione peroxidase (GPx) (Rotruck *et al.*, 1973); reduced glutathione (GSH) (Beutler *et al.*, 1963); hydrogen peroxide (Wolff, 1994); lipid peroxidation (Varshney and Kale, 1990) and ascorbic acid (Jagota and Dani, 1982).

Statistical analysis

All data are expressed as mean ± standard error of mean and were subjected to one-way analysis of variance (ANOVA) using the Graphpad prism 9.0 statistical package. Post-hoc test was carried out using Bonferroni's multiple comparison test.

RESULTS

Table 1 reveals that treatment with EGME only, significantly ($p < 0.05$) reduced the relative organ weight of the liver and the kidneys compared with the control. Co-treatment with P. pinnata at the 50, 75 and 100 mg/kg doses resulted in significant ($p < 0.05$) increase in the relative weights of these organs when compared with the EGME only group and not with the control except at the 25 mg/kg dose.

Table 1. Effect On Relative Organ Weight of Animals Co-Treated with EGME and *P. pinnata*

Dose	Relative organ weight (%)	
	Kidney	Liver
Control	0.74 ± 0.03	4.10 ± 0.24
10% DMSO	0.64 ± 0.10	3.65 ± 0.24
EGME	0.26 ± 0.02[a]	2.11 ± 0.25[a]
EGME + PP (25mg/kg)	0.23 ± 0.02[a]	2.02 ± 0.44[a]
EGME + PP (50mg/kg)	0.72 ± 0.11[b]	3.64 ± 0.42[b]
EGME + PP (75mg/kg)	0.67 ± 0.05[b]	3.62 ± 0.20[b]
EGME + PP (100mg/kg)	0.72 ± 0.09[b]	3.52 ± 0.20[b]

Note: n = 10; a - significantly different from control at $p < 0.05$; b - significantly different from EGME only group at $p < 0.05$

In Table 2, administration of EGME only, significantly ($p < 0.05$) reduced the concentrations of reduced glutathione and ascorbic acid, and significantly ($p < 0.05$) elevated the levels of lipid peroxidation and hydrogen peroxide in the liver in comparison with that of the control. The groups co-administered with P. pinnata at 50, 75 and 100 mg/kg doses had these parameters significantly ($p < 0.05$) reduced compared with the EGME only group, except at the 25 mg/kg dose.

Table 2. The Effect of Co-Administration of EGME and *P. Pinnata* on Certain Antioxidant Parameters in the Liver

DOSE	GSH (µg/mL)	Ascorbic acid (µg/mL)	Lipid peroxidation (MDA formed/ mg protein)	Hydrogen peroxide (µM)
Control	7.42 ± 0.51	21.70 ± 0.78	7.02 ± 0.68	7.29 ± 0.87
10% DMSO	6.50 ± 0.26	21.13 ± 0.75	7.16 ± 0.78	6.71 ± 0.46
EGME (200 mg/kg)	4.25 ± 0.33[a]	14.03 ± 0.81[a]	19.53 ± 0.38[a]	12.14 ± 1.71[a]
EGME+PP (25 mg/kg)	3.79 ± 0.02[a]	13.56 ± 2.50[a]	10.89 ± 0.55[a]	12.36 ± 3.09[a]
EGME+PP (50 mg/kg)	6.65 ± 0.40[b]	19.76 ± 1.12[b]	8.50 ± 0.58[b]	8.09 ± 0.51[b]
EGME+PP (75 mg/kg)	6.49 ± 0.30[b]	17.91 ± 1.00[b]	7.89 ± 0.74[b]	6.72 ± 0.27[b]
EGME+PP (100 mg/kg)	6.57 ± 0.31[b]	20.41 ± 1.49[b]	9.53 ± 0.47[b]	8.40 ± 0.62[b]

Note: n = 10; a - significantly different from control at $p < 0.05$; b- significantly different from EGME group at $p < 0.05$; MDA-malondialdehyde

Figure 1 reveals that administration of EGME only, significantly ($p < 0.05$) reduced the activities of SOD, GST, catalase and glutathione peroxidase in the liver in comparison with the control. The groups co-administered with *P. pinnata* at the 50, 75 and 100 mg/kg doses except at the 25 mg/kg dose, showed significant ($p < 0.05$) elevation of the activities of these enzymes in comparison with the EGME only group.

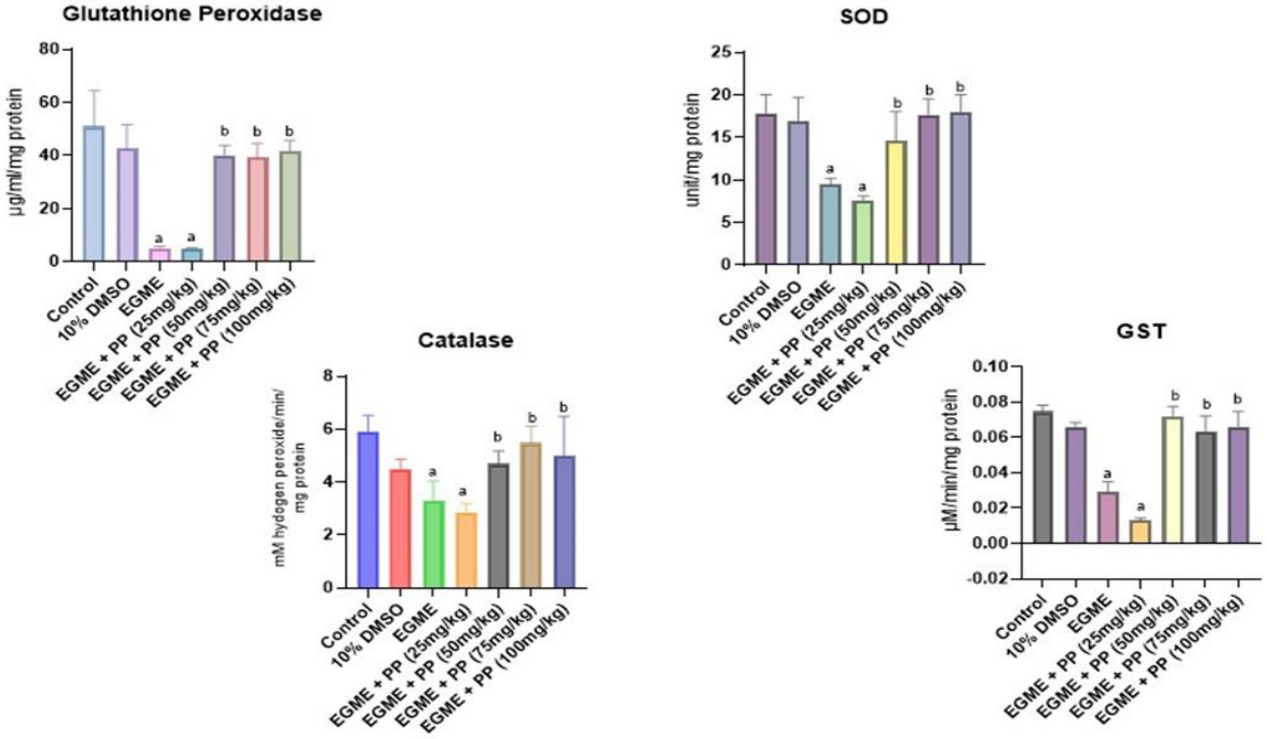

Figure 1. Effect of Co-Administration of EGME And *P. pinnata* on Some Antioxidant Enzymes in the Liver

Note: n= 10; a- significantly different from control at $p < 0.05$; b- significantly different from EGME only group at $p < 0.05$; SOD-superoxide dismutase; GST-glutathione-S-transferase

Table 3. The Effect of Co-Administration of EGME and *P. Pinnata* 0n Certain Antioxidant Parameters in The Kidney

DOSE	GSH (µg/mL)	Ascorbic acid (µg/mL)	Lipid peroxidation (MDA formed/mg protein)	Hydrogen peroxide (µM)
Control	5.79 ± 0.25	35.78 ± 0.82	0.39 ± 0.09	6.36 ± 0.33
10% DMSO	5.62 ± 0.14	33.93 ± 1.25	0.58 ± 0.21	7.43 ± 0.53
EGME (200 mg/kg)	2.21 ± 0.24 [a]	15.72 ± 1.46[a]	1.85 ± 0.27[a]	16.05 ± 0.17[a]
EGME+PP (25 mg/kg)	3.56 ± 0.16 [a]	16.06 ±1.11[a]	1.25 ± 0.06[a]	16.22 ± 0.38[a]
EGME+PP (50 mg/kg)	5.10 ± 0.27 [b]	37.54 ±1.92[b]	0.71 ± 0.03[b]	7.50 ± 0.12[b]
EGME+PP (75 mg/kg)	5.40 ± 0.40[b]	34.20 ± 0.81[b]	0.80 ± 0.03[b]	5.31 ± 0.13[b]
EGME+PP (100 mg/kg)	4.90 ± 0.15[b]	34.39 ± 0.75[b]	0.75± 0.12[b]	7.19 ± 0.19[b]

Note: n=10; a - significantly different from control at $p < 0.05$; b- significantly different from EGME only group at $p < 0.05$; MDA-malondialdehyde

Table 3 reveals that treatment of EGME only, significantly ($p < 0.05$) reduced the concentration of reduced glutathione and ascorbic acid, and significantly ($p < 0.05$) elevated the levels of lipid peroxidation and hydrogen peroxide in the kidney in comparison with the control. Conversely, co-administration with P. pinnata significantly ($p < 0.05$) prevented these effects at the 50, 75 and 100 mg/kg doses except at the 25 mg/kg dose when compared to the EGME only group.

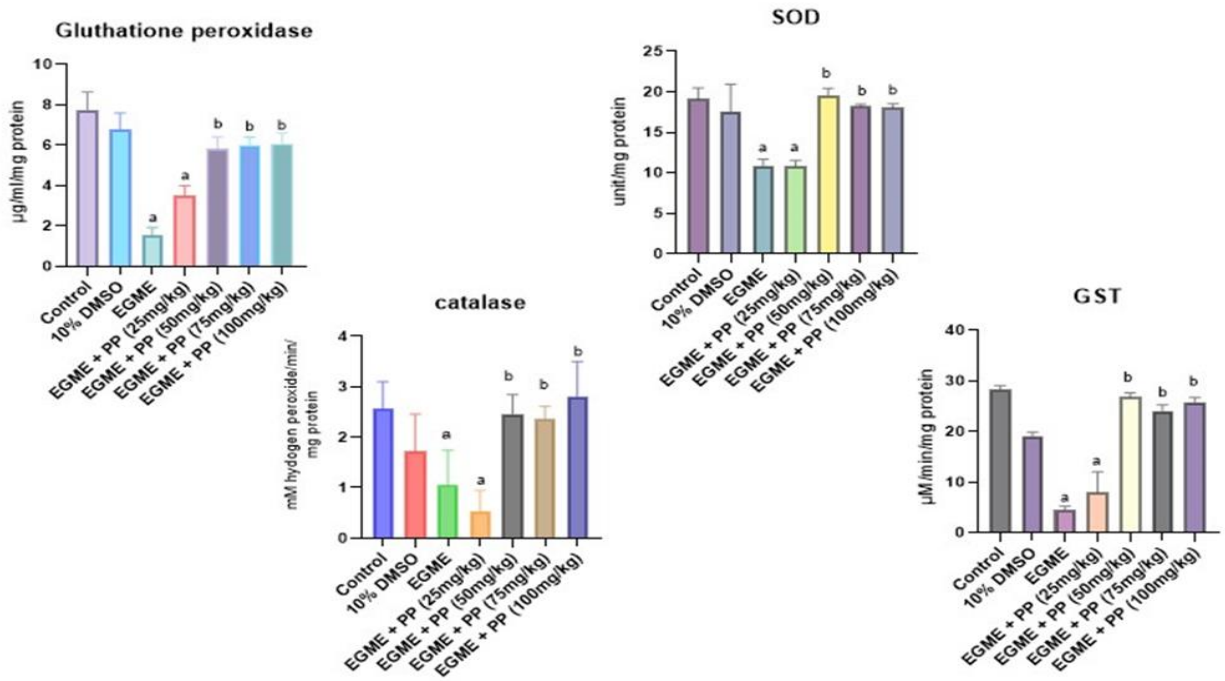

Figure 2. Effect of Co-Administration of EGME and *P. Pinnata* on Certain Antioxidant Enzymes in the Kidney

Note: n= 10; a- significantly different from control at $p < 0.05$; b- significantly different from EGME only group at $p < 0.05$; SOD-superoxide dismutase; GST-glutathione-S-transferase

Administration of EGME only, significantly ($p < 0.05$) decreased the activities of SOD, catalase, GST and glutathione peroxidase in the kidney in comparison with the control. Co-administration with *P. pinnata* significantly ($p < 0.05$) increased the activities of these enzymes at 50, 75, 100 mg/kg doses except at the dose of 25 mg/kg in comparison with the EGME only group (Figure 2).

DISCUSSION

This study showed that the methanol leaf extract of *Paullinia pinnata* prevented the toxic manifestations as a result of exposure to EGME in the liver and kidneys of male Wistar rats at moderate doses of 50, 75 and 100 mg/kg body weight while it had no effect at the dose of 25 mg/kg body weight. The decrease in relative organ weight upon exposure to EGME is a sign of toxicity. This was reversed upon co-administration with P. pinnata at 50, 75 and 100 mg/kg doses but not at the 25 mg/kg dose. Thus, showing that at a low dose, P. pinnata did not prevent the toxic effect of EGME.

Ascorbic acid or vitamin C is a water-soluble vitamin that is known to have antioxidant properties except in the presence of transition metal ions. It is therefore a non-enzymatic antioxidant which effectively reduces reactive nitrogen species, hydrogen peroxide, singlet oxygen, superoxide radical anion and hydroxyl radical (which are all examples of oxidants) (He et al., 2017). In the liver and kidney, the ascorbic acid level in the EGME only treated group and the group co-treated with EGME and 25 mg/kg dose of P. pinnata methanol leaf extract was significantly reduced compared to the control. This suggests that vitamin C is being utilized to combat increased presence of free radicals as a result of insult by EGME on the organs. Hence the observed decrease in its levels in these groups. Also, vitamin C concentration was significantly elevated upon co-treatment with P. pinnata at 50, 75 and 100 mg/kg doses when compared with the EGME only group but not with the control. This implies that P. pinnata methanol leaf extract suppressed the perturbations caused by EGME but not at the 25 mg/kg dose.

Glutathione is a tripeptide which consists of glutamate, cysteine and glycine in peptide linkage. It is the most abundant non-enzymatic antioxidant in the liver and this is the major site of its synthesis. In the reduced form, which is GSH, it plays a crucial role in antioxidant pathways (Chitturi et al., 2021). In the liver and kidney, the EGME only group and the group co-administered with EGME and P. pinnata methanol leaf extract at the dose of 25 mg/kg had significantly reduced concentrations of GSH compared to the control, while that of the groups co-administered with EGME and 50, 75 and 100 mg/kg doses of P. pinnata methanol leaf extract were significantly doused compared to the EGME only group but not with the control. This shows that EGME caused increased release of oxidants which the extract was able to prevent at 50, 75 and 100 mg/kg doses but not at the 25 mg/kg dose. Glutathione peroxidase (GPx) is an enzymatic antioxidant found in a family of enzymes that are related phylogenetically. These are GPx1/GPx2, GPx3/Gpx5/GPx6 and GPx4/GPx7/GPx8. It uses GSH to reduce organic hydroperoxides and hydrogen peroxide to alcohol and water respectively. However, it possesses a higher affinity for hydrogen peroxide than catalase (He et al., 2017; Chitturi et al., 2021). The activity of GPx in the liver and kidney by was diminished in the EGME only and the group co-treated with P. pinnata methanol leaf extract at 25 mg/kg dose when compared to the control but co-treatment with EGME and P. pinnata methanol leaf extract at 50, 75 and 100 mg/kg doses doused this effect. Thus indicating that the aberration as a result of exposure to EGME was prevented by P. pinnata methanol leaf extract at 50, 75 and 100 mg/kg doses but not at 25 mg/kg.

Superoxide dismutase (SOD) is an enzymatic antioxidant that catalyzes the conversion of superoxide into hydrogen peroxide and oxygen. Catalase and GPx then hydrolyze the hydrogen peroxide into water and oxygen (He et al., 2017; Nandi et al., 2019). Activity of SOD in the liver and kidney of the EGME only group and the group co-exposed to EGME and P. pinnata methanol leaf extract at 25 mg/kg dose was reduced compared to the control but was elevated in the groups co-treated with EGME and P. pinnata methanol leaf extract at the doses of 50, 75 and 100 mg/kg compared to the EGME only treated group but not with the control. Again, this suggests that perturbations in the activity of SOD as a result of exposure to EGME was prevented by co-treatment with P. pinnata methanol leaf extract at 50, 75 and 100 mg/kg dose but not at 25 mg/kg dose.

Catalase is one of the most important enzymatic antioxidants and it catalyzes the hydrolysis of hydrogen peroxide to water and oxygen (Kurutas, 2016). Glutathione-S-transferase (GST) is another example of an enzymatic antioxidant which is encoded by a family of large gene and it has multiple functions including detoxification of xenobiotic or endogeneous compounds by conjugating them with GSH and regulating the redox homeostasis in cells (Zhuge et al., 2020). In the liver and kidney of the EGME only group and the group co-administered with EGME and P. pinnata methanol leaf extract at 25 mg/kg dose, the activities of catalase and GST were doused compared to the control while the groups co-exposed to EGME and P. pinnata methanol leaf extract at 50, 75 and 100 mg/kg doses were significantly elevated compared to the EGME only group and not the control. This indicates that the adverse effect of exposure to EGME on the activity of these enzymes was suppressed by co-treatment with P. pinnata methanol leaf extract at 50, 75 and 100 mg/kg doses and not at 25 mg/kg. These observations are supported by the observed elevated levels of lipid peroxidation and hydrogen peroxide in the liver and kidney of EGME only group and the group co-administered with EGME and P. pinnata methanol leaf extract at 25 mg/kg dose, and the reduction in the groups co-exposed to EGME and P. pinnata methanol leaf extract at 50, 75 and 100 mg/kg doses.

Therefore, vitamin C, GSH, GPx, SOD, catalase, and GST are all part of the antioxidant system of the cells that make up the tissues and organs in the body. They scavenge free radicals which damage the DNA, cells, organs and tissues, thus leading to various diseases and health challenges (He et al., 2017; Nandi et al., 2019). The activities and levels of these antioxidant parameters were reduced in the EGME only group and the group co-treated with EGME and P. pinnata at 25 mg/kg dose in the kidney and in the liver, thus buttressing the already established documentation on the toxic

manifestations of EGME (Adeyemo-Salami, 2021). This observation has been reported to occur in disease conditions (Nandi et al., 2019). However, these were barred by co-treatment of EGME with P. pinnata at 50, 75 and 100 mg/kg doses, thus showing that P. pinnata was effective in preventing the deleterious effect of EGME on the antioxidant defense system of the liver and the kidney at these doses. This observation is also similar to that of Elguindy et al. (2018) who demonstrated that treatment with diethylnitrosamine lowered the level of reduced glutathione and activities of the antioxidant enzymes in the kidney of Sprague Dawley rats but this was ameliorated by treatment with the essential oil of Elettaria cardamomum. This is also akin to the report of Ibrahim et al. (2019) using black berry juice which reversed the decrease in the activities of the antioxidant enzymes by acrylamide both in the liver and kidney.

CONCLUSION

Taken together, Paullinia pinnata possesses the capacity to circumvent the insult by ethylene glycol monomethyl ether in the liver and kidneys at moderate doses. It would therefore be useful when ingested to prevent and maybe combat the toxic effects of ethylene glycol monomethyl ether in those organs. Therefore, Paullinia pinnata would be useful for persons who are exposed to ethylene glycol monomethyl ether when ingested moderately. Moreover, the active principle(s) can be isolated and packaged into drugs or food supplements.

AUTHORS' CONTRIBUTIONS

Conceptualization, OAA; methodology, OAA; validation, OAA; formal analysis, DSK and LOA; investigation, DSK and LOA; resources, OAA, DSK and LOA.; data curation, OAA; writing—original draft preparation, OAA; writing—review and editing, OAA; supervision, OAA; project administration, OAA; funding acquisition, OAA, DSK and LOA. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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