Retraction Notice

Title of retracted article:	**Effects of Annona squamosa Seed Extract on Mus musculusExposed to Mercuric Chloride: Evaluations of Metal-Induced Oxidative Stress and Its Developmental Outcomes**
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Retraction initiative (multiple responses allowed; mark with **X**):

☐ All authors
☐ Some of the authors:
X Editor with hints from
- Journal owner (publisher)
- Institution:
- **X** Reader: Joycelyn C. Jumawan
- Other:

Retraction type (multiple responses allowed):

☐ Unreliable findings
- Lab error
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History
Expression of Concern:
☐ yes, date: yyyy-mm-dd
X no

Correction:
☐ yes, date: yyyy-mm-dd
X no

Comment:

The article has been retracted due to the investigation of complaints received against it. The substantial portions of the text came from Dr. Joycelyn C. Jumawan, " Effects of *Annona squamosa* leaf extract on *Mus musculus* exposed to mercuric acetate: Evaluations of metal-induced oxidative stress and its developmental outcomes ". The scientific community takes a very strong view on this matter and we treat all unethical behavior such as plagiarism seriously.

This article has been retracted to straighten the academic record. In making this decision the Editorial Board follows COPE's Retraction Guidelines. Aim is to promote the circulation of scientific research by offering an ideal research publication platform with due consideration of internationally accepted standards on publication ethics. The Editorial Board would like to extend its sincere apologies for any inconvenience this retraction may have caused.

Editor guiding this retraction: Dr. Sukumar Saha(EiC of AJPS)

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Effects of *Annona squamosa* Seed Extract on *Mus musculus* Exposed to Mercuric Chloride: Evaluations of Metal-Induced Oxidative Stress and Its Developmental Outcomes

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Abstract

The effects of ethanolic extract from the seeds of *Annona squamosa* were studied in gestating ICR mice subcutaneously treated with 4 mg/kg BW mercuric chloride (MA) and their fetuses *in utero* starting GD 14 - 16. Effects of solo exposure of *A. squamosa* at 25 mg/kg BW or its combinatorial exposure at 25 mg/kg BW and 75 mg/kg BW with MA in the maternal mice and their fetuses were evaluated at GD 19. Results showed that MA significantly increased maternal mortality and fetal resorption index, while inducing decreased fetal size. Exposure of MA and the two seed extract doses significantly enhanced this effect. *A. squamosa* at 25 mg/kg BW induced abortion and resorption of embryos at a lesser extent compared with the two doses with MA. Lipid peroxidation assay revealed lowered MDA values in the liver and brain of maternal mice exposed to solo and combinatorial exposure of *A. squamosa* with MA, but not with their fetal counterpart. Maternal mice and fetuses exposed to the two *A. squamosa* doses with MA show marked hepatic and neurologic histological injury similar to the positive control. The results suggest that *A. squamosa* seeds have the potential to protect against mercury-induced oxidative stress in the maternal mice, but may not be appropriately applied in gestating subjects due to its potential abortifacient property.

Keywords

***Annona squamosa* Seeds, Oxidative Stress, Mercuric Chloride**

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1. Introduction

Sulfhydryl-reactive metals like mercury can promote the formation of hydrogen peroxide and enhance the subsequent iron and copper-induced production of lipid peroxides and the highly reactive hydroxyl radicals [1]. These metals disrupt the structure and function of numerous important proteins through direct binding to free sulfhydryl groups, increasing the resistance to the metal by scavenging reactive oxygen species (ROS) [2].

Apart from leaking through the placental barrier, mercury can also be concentrated in the brain of the developing fetus, because the metal is absorbed quickly and is not excreted efficiently resulting in teratogenesis in affected embryos [3]. It is a potent neurotoxicant that can affect the fetus, so that even low and medium prenatal and postnatal exposure to methylmercury (MeHg) can result in locomotor, motor coordination and learning deficits [4]. Mercury can also affect the detoxifying activity of the liver resulting to various forms of hepatoxicity [1] [5].

Annona squamosa Linn. locally known as sugar apple in the Philippines is widely popular for its edible fruit and for its use as a powerful pesticide [4] [6] [7]. Lately, the ethanolic extracts of its seed have been reported to have hypoglycaemic and anti-diabetic effects [8]-[10]. *In vitro* studies utilizing ethanolic and flavonoid positive fractions have shown that its seeds have antioxidant, chemopreventive and chemotherapeutic activities [11] with flavonoids exhibiting the highest inhibitory activity when assayed for the ability to scavenge the diphenylpicrylhydrazyl (DPPH) free radical [12]. The water extract of *A. squamosa* seeds possessed antioxidant activity as shown by increased activities of scavenging enzymes, catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione reductase (GR) and glutathione-stransferase (GST), and decreased malondialdehyde levels in various organs of Winstar rats [8]. Murihexocin C, an acetogenin isolated from its seeds, was found to induce apoptosis in human colon carcinoma Col2 cell line [13].

Antioxidants provide protection to living organism from damage caused by uncontrolled production of ROS-concomitant lipid peroxidation, protein damage and DNA strand breaking [1] [13]. These interfering effects of anti-oxidants on the damaging effects of sulfhydryl reactive metals have significant consequences on the developing embryo. It has been proposed that plants with potent anti-oxidant properties may also have the potential to interfere, and hence mediate the effects of heavy metal toxicity [2] [14].

The possible maternal-fetal protective attenuation of *A. squamosa* ethanolic seed extract against mercury-induced oxidative stress during gestation has not been explored so far. Further, possible effects of the extract to minimize congenital hepatic and neurotoxic effects of methyl mercuric chloride on fetus exposed *in utero* can offer new insights to its protective function on embryonic development, in addition to its myriad of known therapeutic effects; hence, this study will elaborate above aspects.

2. Materials and Methods

2.1. Preparation of Seed Extract

Mature seeds of *A. squamosa* were collected from the forests of Morong, Bataan and later processed at the Institute of Biology, University of the Philippines. Seeds were deveined, rinsed and shade-dried until crispy. Forty five (45) grams of the dry, powderized material was immersed in 95% analytical grade ethanol for 48 hours. The mixture was filtered and evaporated to dryness using a rotary evaporator. The resulting product was lyophilized until in powder form. Powderized form of the ethanolic extract was reconstituted in distilled deionized water to make a stock solution of 25 mg/mL and 75 mg/mL (Dosage based on Gupta *et al*., 2008 [8]).

2.2. Test Agents

Distilled deionized water was used as negative control treatment. 4 mg/kg of mercuric chloride (MA) (Fisher Scientific Company) was used as positive control [5]. A solo 25 mg/kg BW of ethanolic seed extract from *A. squamosa* was used to test the independent effect of the extract. Two other extract doses (25 mg/kg BW and 75 mg/kg BW) of *A. squamosa* were used in combinatorial treatment with 4 mg/kg BW mercuric chloride (MA) as test treatments.

2.3. Animal Stock

Fifteen (15) adult breeder males (>6 months old; 28 - 33 g) and thirty six (36) female (8 weeks old; 28 - 31 g)

ICR strain white mice (*Mus musculus*) purchased from Bureau of Food and Drugs, Animal Research Division were used in this study. Mice were fed with standard high protein pellets and water ad libitum and maintained under laboratory conditions (temperature 26˚C ± 2˚C, 12 h natural light/dark cycle).

2.4. Breeding and Treatment Set-Up

Adult females were initially induced to estrus 72 hours prior to exposure to males. Females undergoing estrus were then randomly paired overnight with vigorous sexually experienced males in 2 female: 1 male ratio per cage. Successful mating was confirmed by the presence of vaginal plugs, the observance of which was considered as gestational day 0 (GD 0) of pregnancy. Subjects were randomly partitioned into 6 groups with 6 vaginal plug-positive females as replicates and treated as follows: Group 1: distilled water *ad libitum* (negative control 1; -C1); Group 2: subcutaneous injection of distilled deionized water at 10 mL/kg (negative control 2; -C2); Group 3: 4 mg/kg BW mercuric chloride in distilled deionized water medium (MA); Group 4: ethanol seed extract of *A. squamosa* (ELEAS) at 25 mg/kg BW; Group 5: 25 mg/kg ethanolic seed extract of *A. squamosa* and 4 mg/kg BW mercuric chloride; Group 6: 75 mg/kg ethanolic seed extract of *A. squamosa* and 4 mg/kg mercuric chloride. The extracts and vehicles were given subcutaneously for 3 consecutive days starting at GD 14 as gestation is already prominent and passage of mercuric chloride in the mice placenta is enhanced in this stage. Each of the treated females was sacrificed on GD 19 and the number of viable and resorbed fetus and resorption sites was recorded.

2.5. Gross Anatomical Examination, Morphometry and Isolation of Vital Organs

Pregnant mice were weighed daily in order to monitor weight changes. On GD 19, isolated fetuses were examined for morphologic abnormalities such as hydrocephalus, oedema, cleft lip and palate. The number of fetuses in the uterus/dam were recorded and their corresponding weights taken. Percent embryonic loss after implantation was calculated as: (*number of implantations-number of fetuses in development/ number of implantations)* × 100 and used as a measure of toxicity effect [15]. Mortality rate of pregnant mice during the course of treatment per group was calculated using the formula: *Mortality rate* (*Number of deaths/Treated females*) × 100.

Morphometry of individual fetuses were measured using a digital caliper. The following indices were measured—*crown-rump length* (*CRL*) as the measurement from the skull vertex (crown) to the midpoint between the apices of the buttocks (rump); *head-tip length* (*HdL*) as the measurement from the back of the head to the tip of the recognizable lip, *forelimb length* (*FL*) as the measurement between the top and the tip of the forelimbs and *hind-limb length* (*HL*) as the measurement between the top and tip of the recognizable hind limb.

2.6. TBARS Assay for Lipid Peroxidation

Lipid peroxidation assay of the liver and brain of the maternal mice and their fetuses were done following the procedure of Ohkawa *et al*. (1979) [16] with certain modifications. Liver and brain samples were isolated and processed rapidly after sacrifice at GD 19. Samples were weighed and homogenized in a glass tissue grinder using 0.05 M phosphate buffer solution to yield a concentration of 100 mg net tissue weight per mL of the homogenizing medium. 0.5 mL of the homogenate was added with 2.5 mL trichloroacetic acid (TCA) and 1 mL of thiobarbituric acid (TBA) and the resulting mixture vortexed. Test tubes containing the mixture were placed in boiling water (100˚C) for 30 minutes, cooled to room temperature and added with 4 mL n-butanol. Mixture was vortexed and the n-butanol layer was centrifuged at 3300 rpm and 25˚C for 10 minutes. Absorbance of the organic layer was measured spectrophotometrically at 535 nm.

2.7. Histological Preparation and Microscopic Observation

Brain and liver from the maternal and fetal samples were fixed in 10% buffered formalin and subjected to routine histological preparation. Three sections from three maternal and fetal samples for each treatment group were examined using a light microscope. Histology of cross sections from the cerebral cortex, cerebellum and hippocampus were done to assess neurotoxicity of the brain. % of occurrence of any form of pathology or lesion was noted for each treatment group. Liver hyperplasia was scored manually by counting the number of nuclei in a liver area of 25,500 μm^2 (mean of 5 counts/ section). Frequency of occurrence of necrotic, hypertrophic cells or steatosis per replicate section were noted.

2.8. Statistical Analysis

Parameters in percentages (%)—mortality rate, and % resorption were subjected to non-parametric Kruskall-Wallis test to determine dose or treatment response. Analysis of variance (ANOVA) was utilized to determine differences between treatment groups for values such as maternal weight gain, fetal size, fetal weights, morphometric measurements and malondialdehyde (nmol protein-1) values. When the ANOVA revealed significant differences among treatment groups, Duncan's multiple range test (DMRT) was utilized to pinpoint specific treatment differences. Differences were considered statistically significant if ≤ 0.05.

3. Results

3.1. Mortality and Gross Morphologic Observations

Resorptions of fetuses in the MA-treated pregnant mice were noted even prior to GD 19. Uteri of the dams were seen with resorbed embryos or resorbed sites and swellings that marked previous implantations (Figure 1). Some representative samples treated with MA and combinations with ELEAS had 100% resorption of embryos. Maternal mortality in mice treated with solo ELEAS is significantly lower than those treated with MA or in combination of the two doses (Figure 2(b)). Nonetheless, this relatively "improved" response is still significantly lower compared to the negative controls across all indices (<0.05) (Table 1).

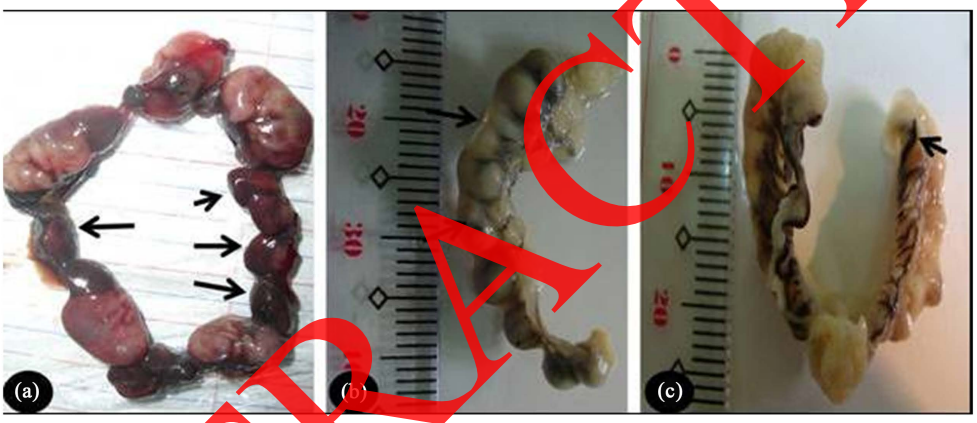

Figure 1. (a) Resorbed embryos (arrows) in a female treated with 4 mg/kg MA (GD 17); (b) 100% fetal resorption in pregnant mice treated with MA + ELEAS (GD 17); (c) 100% resorption in a dam treated with MA + 75 mg/kg ELEAS.

Table 1. Reproductive indices/outcomes in the maternal and fetal *Mus musculus* exposed to varying concentrations of ethanolic seed extract from *A. squamosa* (ELEAS) and mercuric chloride (MA) and their combinations.

Indices	Maternal Wt. gain (g)	Fetal size (n)	Fetal Wt. (g)	Resorption index (%)	% death	CRL (mm)	HdLL (mm)	FL (mm)	HL (mm)
-Control 1	14.50 ± 1.65^{a}	10.82 ± 1.64^{a}	2.65 ± 0.17^{a}	0	0	22.9 ± 0.71^{a}	11.28 ± 0.87^{a}	7.43 ± 0.40^{a}	7.3 ± 0.70^{a}
-Control 2	16.83 ± 2.13^{a}	11.3 ± 1.21^{a}	1.94 ± 0.08^{a}	5.14	0	20.12 ± 1.55^{a}	11.12 ± 0.53^{a}	9.26 ± 0.12^{a}	8.1 ± 0.05^{a}
4 mg/kg MA	5.21 ± 1.91^{c}	4.31 ± 1.70^{c}	1.1 ± 0.024^{b}	52	51	16.2 ± 1.27^{b}	8.61 ± 1.79^{b}	7.13 ± 0.53^{b}	6.5 ± 0.21^{b}
25 mg/kg ELEAS	12.73 ± 2.16^{a}	8.2 ± 2.10^{b}	1.68 ± 0.17^{b}	45	21	14.1 ± 0.96^{b}	8.23 ± 0.41^{b}	6.16 ± 0.44^{b}	6.1 ± 0.15^{b}
MA + 25 mg/kg ELEAS	7.316 ± 1.64^{b}	6.13 ± 1.75^{c}	1.18 ± 0.15^{b}	57	37	11.07 ± 0.45^{c}	7.86 ± 0.26^{b}	5.16 ± 0.89^{b}	2.73 ± 0.27^{c}
MA + 75 mg/kg ELEAS	6.88 ± 2.14^{b}	4.13 ± 1.87^{c}	1.2 ± 0.03^{b}	48	53	11.56 ± 0.39^{c}	7.22 ± 0.35^{b}	6.31 ± 0.37^{b}	5.29 ± 1.02^{c}

Means with different letters (columns) are significantly different from each other ($p < 0.05$).

Comparison of morphometric variables reveal that fetuses treated with MA and the two doses of ELEAS are significantly smaller than the negative control (**Figure 2(a)** and **Figure 3**). However, no abnormal phenotypes such as cleft palate or cleft lip, or deformed digits were observed in fetuses gestationally exposed to solo ELEAS. Abnormal swellings/outgrowth in the neck portion of embryos in the simultaneous exposure of MA + 75 mg/kg ELEAS were observed (**Figure 3(j)**).

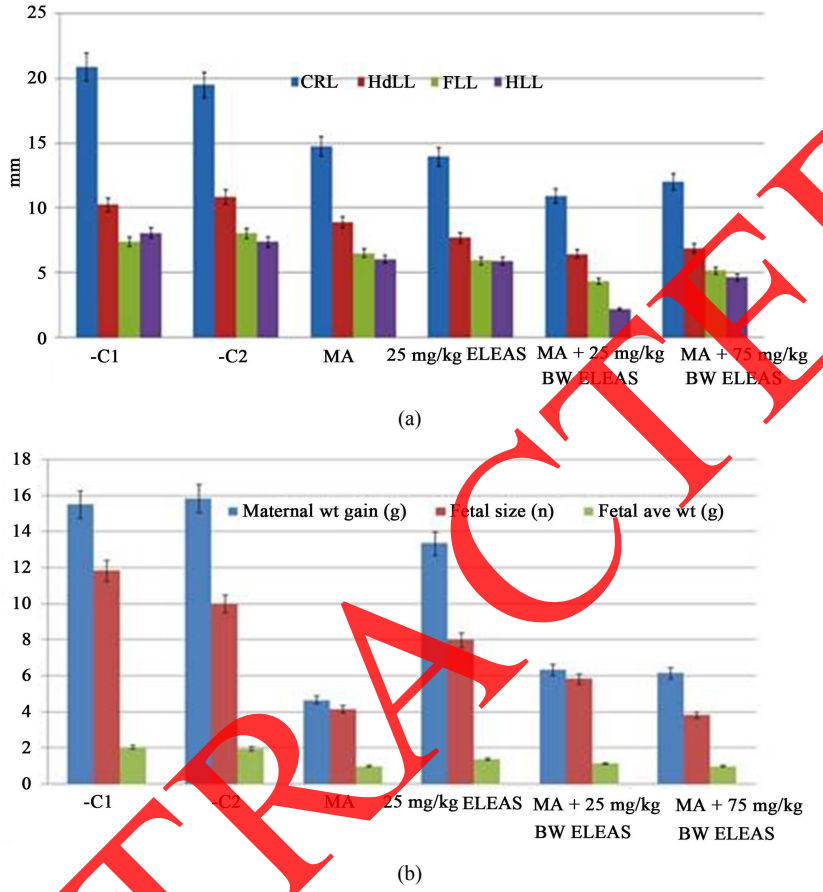

Figure 2. Morphometric (a) and reproductive (b) indices of maternal and fetal *Mus musculus* exposed to ethanolic seed extract from *A. squamosa* (ELEAS). CRL-Crown rump length, HdLL-Head lip length, FLL-Forelimb length, HLL-Hindlimb length, ELEAS-ethanolic seed extract from *A. squamosa.*

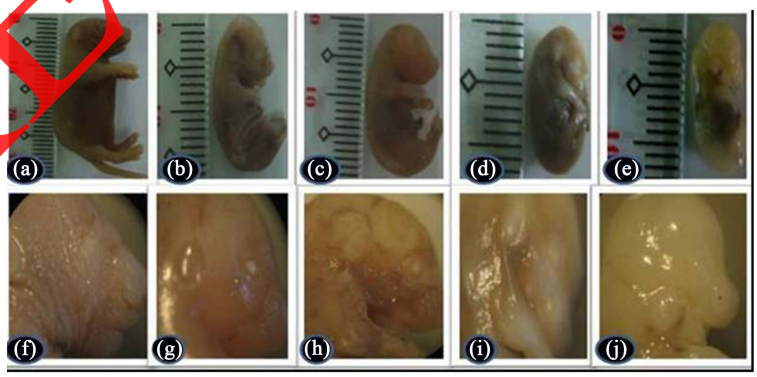

Figure 3. Effects of *A. squamosa* extracts on the external morphology of fetal *Mus musculus.* (a)-(f) Control; (b)-(g) Fetus exposed *in utero* to 4 mg/kg bw MA; (c)-(h) Fetus exposed *in utero* to 25 mg/mL bw ELEAS; (d)-(i) Effects of *in utero* exposure to MA + 25 mg/kg bw ELEAS; ((e), (j), (o), (t)) of *in utero* exposure to MA + 75 mg/kg ELEAS.

3.2. Lipid Peroxidation Assay

Overall comparison of oxidative stress response shows that all treatments have significantly higher MDA (nmol mg·protein^{-1}) levels compared to the negative controls (**Figure 4** and **Table 2**). Comparison however between maternal-fetal brain and liver MDA levels show that maternal mice treated with ELEAS have significantly higher MDA values ($p < 0.05$) compared to their fetuses where MDA levels were not significantly different from the positive control (>0.05). MDA levels of the fetal brain and liver in the solo treatment of 25 mg/kg ELEAS were significantly smaller compared to those gestationally exposed to MA. MDA values of the latter two treatments were significantly smaller than MA ($p < 0.05$).

3.3. Liver Histology

Maternal liver exposed to single and combinatorial treatments of MA and ELEAS show varying histologic injury compared to the negative controls (**Figure 5**). Maternal liver exposed to MA alone showed consistent dissociation of often necrotic hepatocytes (**Figure 5(c)** and **Figure 5(d)**). This pathological observation seemed to be enhanced with the subsequent treatment of 25 and 75 mg/kg ELEAS where vacuolations and massive steatosis were observed (**Figure 5(e)** and **Figure 5(f)**). Solo treatment of 25 mg/kg ELEAS also caused minimal incidence of pathologies in the liver (30%). Combinatorial treatment of MA and ELEAS resulted in mixed lymphomonocytic infiltrations as well as formation of cell aggregates (**Figure 5(g)** and **Figure 5(h)**).

Observations of histologic injury in the fetal liver were higher (67%) than in the maternal counterpart (34%). Hepatocellular necrosis and abundance of neutrophils near terminal hepatic venules in the fetal liver exposed *in utero* to MA are of common occurrence in many samples (**Figure 6(d)** and **Figure 6(f)**). Steatosis, vacuolations and invasion of megakaryocytes and neutrophils especially near venules and extravasation of red blood cells were consistently abundant in combinatorial treatments of MA with the two doses of ELEAS (**Figure 6(c)** and **Figure 6(d)**). Erythroid and myeloid precursors were seen abundantly in livers exposed to combinatorial treatments than in the negative control.

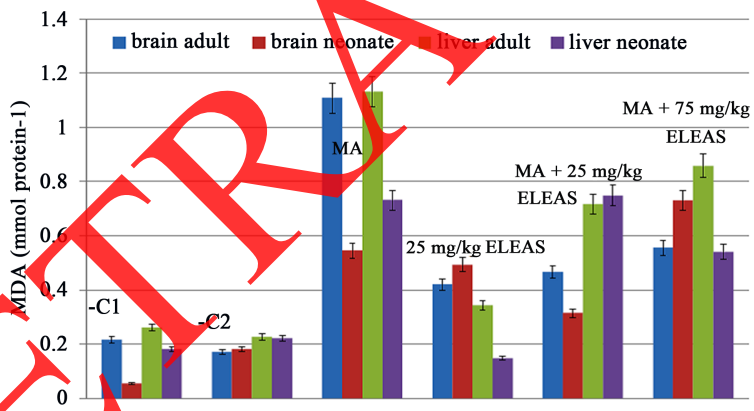

Figure 4. MDA (nmol protein-1) levels through lipid peroxidation assay in maternal and fetal brain and liver.

Table 2. MDA (nmol protein-1) levels through lipid peroxidation assay in maternal and fetal brain and liver.

Treatment	Brain maternal	Foetal	Liver maternal	Foetal
-Control 1	0.311 ± 0.16^{c}	0.058 ± 0.36^{d}	0.262 ± 0.20^{d}	0.191 ± 0.62^{c}
-Control 2	0.191 ± 0.01^{c}	0.184 ± 1.31^{d}	0.278 ± 0.81^{d}	0.316 ± 0.12^{c}
4 mg/kg MA	1.30 ± 0.011^{a}	0.594 ± 1.2^{b}	1.210 ± 1.11^{a}	0.716 ± 0.56^{a}
25 mg/kg ELEAS	0.420 ± 0.18^{b}	0.51 ± 0.52^{c}	0.384 ± 0.75^{c}	0.178 ± 0.57^{c}
MA + 25 mg/kg ELEAS	0.466 ± 0.12^{b}	0.378 ± 0.32^{c}	0.895 ± 0.11^{b}	$0.772 \pm 0.77a$
MA + 75 mg/kg ELEAS	0.555 ± 0.08^{b}	0.791 ± 0.71^{a}	0.921 ± 0.39^{b}	0.589 ± 0.32^{b}

Means with different letters (columns) are significantly different from each other ($p < 0.05$).

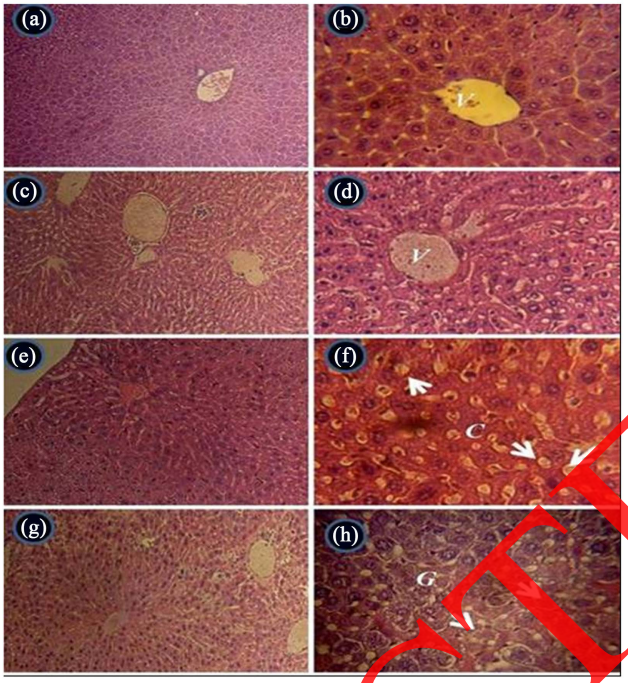

Figure 5. Maternal liver exposed to solo and combinatorial treatments of MA and ELEAS. (a)-(b) Liver from negative control; (c)-(d) Maternal liver exposed to MA; (e)-(f) Maternal liver treated with 25 mg/kg ELEAS; (g)-(h) Maternal liver exposed to combinatorial treatment of MA and ELEAS; (c) White arrows in F-fatty exchange; (g) White arrows in (h) aggregates; ((h) and (e) stain; (a) (c) (d) (g) = 100×; (b) (d) (f) (h) = 400×).

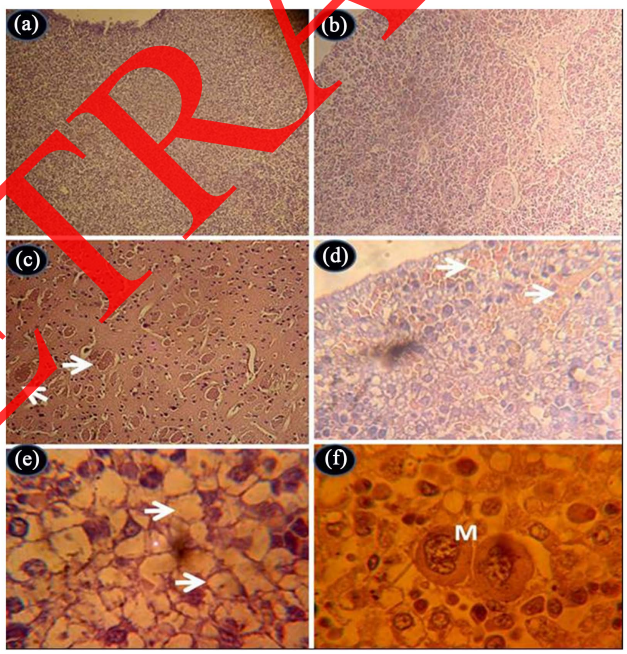

Figure 6. Fetal liver exposed in utero to single and combinatorial treatments of MA and ELEAS. (a) Liver from negative control; (b) Fetal liver gestationally exposed to 25 mg/kg ELEAS; (c)-(d) Combinatorial exposure of MA + 25 mg/kg ELEAS; (c) Clumping of cellular mass in some hepatic lobules (white arrows); (d) Extravasation of red blood cells (white arrows); (e)-(f) Combinatorial exposure of MA + 75 mg/kg ELEAS; (e) Steatosis and vacuolations (white arrows); (f) Invasion of megakaryocytes (M); ((h) and (e) stain; (a)-(b) = 100×; (c)-(d), (f) = 400×; (e) = 1000×).

3.4. Brain Histology

MA exposure also caused decreased density of neurons in the hippocampus of the maternal mice (**Figure 7**). Observations of cerebellar sections in the maternal brain exposed to MA reveal consistent hemorrhagic lesion in the white matter which was also consistently observed in the MA + 75 mg/kg ELEAS combination (**Figure 7(b)** and **Figure 7(c)**). Distinct vacuolations, irregularly dispersed purkinje cells and occurrence of neoplastic aggregation of cellular mass in the combinatorial exposure were also observed in the maternal brain (**Figure 7(f)**).

In utero exposure of the fetus to MA and combinations of low and high doses of ELEAS destroyed the cellular integrity in the fetal brain (**Figure 8**). Extensive loss and degeneration of neurons exposed to MA (**Figure 8 (b)**) and pronounced disintegration and pyknosis in combination treatments of MA and ELEAS at 25 mg/kg and

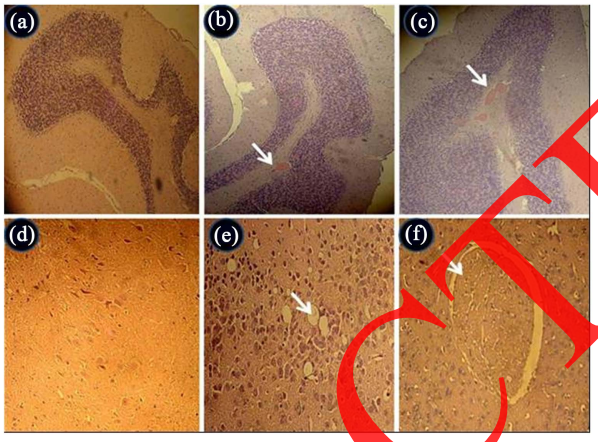

Figure 7. Portions of the maternal brain exposed in to single and combinatorial treatments of MA and ELEAS. (a)-(c) Representative section at the region of the rostral cerebellum; (a) Normal distinctive architecture between the granular and molecular layers; (b)-(c) Hemorrhagic lesion in the white matter (white arrow) in a cerebellar section exposed to MA, consistently observed in the MA + 75 mg/kg ELEAS; (d) Cerebrum showing normal histology; (e) Defined vacuolations and irregularly dispersed purkinje in the same region of a brain exposed to MA; (f) Frequent observations of neoplastic aggregation of cellular mass (white arrows) in the combinatorial treatments of MA + ELEAS. ((h) and (e) stain; (a)-(c) = 100×; (d)-(f) = 400×).

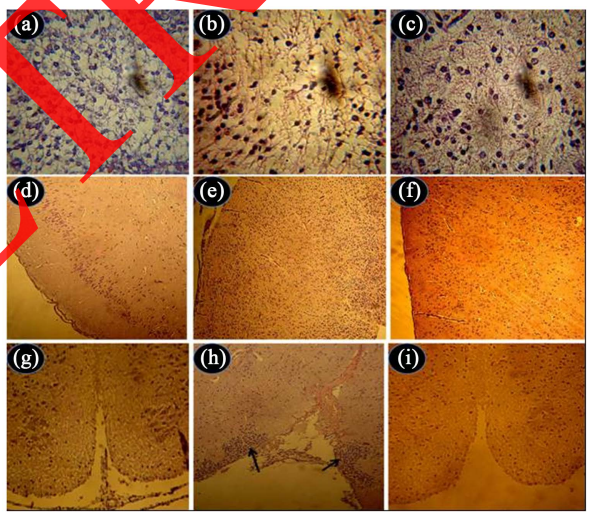

Figure 8. Portions of the fetal brain gestationally exposed to single and combinatorial treatments of MA and ELEAS.S. (a)-(c) Cross section of the cerebellum; (a) Intact and proportioned layers in the negative control; (b) section from brain exposed to MA+ ELEAS; (e)-(f) Irregular aggregation of neurons in the pia matter exposed to MA and combinatorial treatments of MA + 25 mg/kg bw (f) compared to the negative control (d); G-I Sections along the pia and arachnoid layers of the brain meninges. All treatments caused reduction and disintegration of neurons near the posterior region ((h)-(i)); ((h) and (e) stain; (a)-(c) = 400×; (d)-(i) = 100×).

75 mg/kg were consistently observed (**Figure 8(c)**). Frequent observations of abnormal lesions along with mild and severe vacuolations of cells in fetal brain exposed *in utero* to low and high combinations of ELEAS with MA were also observed (**Figure 8(h)** and **Figure 8(i)**). Occurrence of pathology in the fetal brain is more frequent (77%) compared to the maternal brain (51%).

4. Discussion

The present study evaluated the effects of ethanolic seed extract from *A. squamosa* in mercuric chloride-induced oxidative stress and the possible attenuation of the antioxidant properties of the extract in the reproductive response and maternally-relayed effects to the developing liver and brain of the fetus.

The adapted dosage of *A. squamosa* (25 mg/kg and 75 mg/kg) from antidiabetic studies [8] was shown to cause toxicity and some incidence of fetal resorption in this study. This observation may indicate that this dosage may not be appropriate during gestation and that *A. squamosa* may have a potential abortifacient effect if treated at GD 14 onwards in mice. The seed extract of *A. squamosa* at high concentrations (300 - 600 mg/kg BW) did not interfere with the reproductive performance of pregnant rats exposed at GD 10 [17]. Mishra *et al*. (1979) [18] once noted that an active lipid-soluble annonin in the seeds might be the active component responsible for the abortive and anti-implantational activities when given to pregnant rats.

No distinct form of teratogenesis however was observed with exposure to the plain seed extract of *A. squamosa*, so it was likely that the malformations observed in the combinatorial exposure of MA + ELEAS may have been caused by MA. Oral exposure of mercuric chloride at a lower dose (0.25 - 1.0 mg/kg) produced adverse effects on the reproductive performance of mice in the absence of overt mercury toxicity [15]. Pregnant hamsters gavaged with a single 22 mg/kg of mercuric chloride at GD 8 resulted in increased incidence of resorption of embryos [19] while dose-related reduction of mean litter size resulted from treatment of male rats with 1, 2.5, or 5 mg of mercury/kg/day of methyl mercuric chloride prior to mating [20].

Oxidative stress has been pointed out as an important molecular mechanism in methylmercury (MeHg) intoxication [13] [21]. The hazardous effects of MeHg are well known and seem to be related to thiol depletion that, in turn, can lead to an increase in intracellular oxidative stress [22]. Combinatorial treatments of MA and *A. squamosa* that showed significantly reduced MDAs reflect that it was able to counteract the oxidative stress induced by MA exposure. This result affirms the antioxidant activity as reported by Gupta *et al*. (2008) [8]. Significant finding in this aspect of the present study therefore suggest that *A. squamosa* may have the potential to rescue the maternal liver and the brain from oxidative stress, however, possible attenuation in combinatorial treatments should not be applied in gestating females as it may not be able to reduced oxidative stress and it can in fact cause premature farrowing, abortion and resorption a certain degree therefore compromising the development of the fetus at the expense of protecting against the maternal metal-induced toxicity.

Ercal *et al*. (2001), Goyer (1990) and Yoshida (2002) [13] [23] [24] noted that the placenta can enhance the passage of mercury through this barrier system. However, metal-binding protein metallothionein may also play a significant part in this response, hence could be explored in future studies. Enhanced MDA in the fetal brain may be due to the "still developing" blood brain barrier [25] which might not be fully functional during the time of *in utero* exposure to mercury. This probable unregulated passage of mercury in the fetal brain may have profound adverse effects later. Prenatal exposure to MeHg disrupts the postnatal development of the glutathione antioxidant system in the mouse brain, pointing to an additional molecular mechanism by which MeHg induces pro-oxidative damage in the developing CNS and the liver [26].

The results of the present study reinforce that MA-mediated oxidative stress plays a key role in metal-induced neurotoxicity. The hippocampal pathology observed in this study upon exposure is a common manifestation of mercury-induced toxicity [2]. Cognitive studies of prenatally exposed mice to MA were shown to have spatial and cognitive defects that may persist throughout life [26]. Chronic intra-uterine exposure to low-dose MeHg induces a decrease in neuron population in the limbic system, and the offspring may have impaired higher brain function [27].

5. Conclusion

A. squamosa ethanolic seed extract might attenuate mercury-induced toxicity in the maternal liver and brain, but not in the fetal brain and liver which could also explain the enhanced histopathologic injury in the fetal brain and liver compared with its maternal counterpart. Nonetheless, this antioxidant capacity of the extract was not able

to protect against the histopathologic injury in the maternal brain and liver of the treated subjects. The intriguing facet of the extract—the protective capacity of the extract against metal induced oxidative stress, as well as its potential to be abortifacient in gestating mice, requires further studies to elucidate if these two facets are separate mechanisms and the active agents in this plant responsible for such developmental outcomes. The anti-oxidizing mechanism and toxicological implications of using *A. squamosa* may be explored in future research using other metal toxicants aside from mercury. It is recommended that future studies should explore lower doses and the extract at earlier stages of gestation. The postnatal physiologic and cognitive/behavioral features of neonates after being exposed to mercuric chloride *in utero* may be explored in long term studies.

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