Flaxseed alleviates toxic effects of some environmental pollutants on pregnant rats and their foetuses

Abdelgawad Ali Fahmi¹; Mohamed Aly El-Desouky¹; Khairy A. Ibrahim² and Hala Abdelazeem Abdelgaid¹

¹Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt.
²Mammalian Toxicology Department, Central Agriculture Pesticides Lab, Agriculture Research Center, Dokki, Giza, Egypt.

*Correspondence: halaabdellahman1985@gmail.com Accepted: 05 July 2018 Published online: 13 Aug. 2018

Natural exogenous antioxidants can protect pregnant women and their fetuses against toxicity of environmental pollutants exposure especially those can pass through placental barrier. So, this study objective to evaluate if Egyptian flaxseed (FS) whole grain can protect pregnant rats and their foetuses against diesel exhaust particles (DEPs) and/or fenitrothion (FEN) toxicities or not. A total of 48 timed pregnant rats were classified into eight groups (n=6); control, flaxseed (FS), three intoxicated groups and three treated groups. Intoxications were done by an intranasal installation of DEPs (1.5 mg/kg/day) and/or an oral gavage of FEN (1/200 of LD50= 3.76mg/Kg/day) from gestational day 6 to 19. The treatments were done by supplementation the diet of rats with 15% flaxseed from first day of gestation and intoxicated with DEPs and/or FEN from 6 day of gestation. At gestational day 19th the pregnant rats were slightly anaesthetized and the blood samples were collected for biochemical parameters measurements, then animals were sacrificed and uteri were collected, weighted, implantation sites, placental weight foetal number, weight, length, mortality rate and morphological abnormalities were recorded. This study showed that DEPs and/or FEN intoxication caused marked disturbances in maternal biochemical profile as compared with control, foetal growth retardation and many external abnormalities were also observed. Flaxseed supplemented-pregnant rats showed amelioration in biochemical profile as well as foetuses health as compared with parallel intoxicated ones. Thus flaxseed supplementation protects pregnant rats and their fetuses from toxicological effects induced by DEPs and/or FEN exposure.

Keywords: Flaxseed, Diesel Exhaust Particles, Fenitrothion, Pregnancy, Foetuses, oxidative stress, Enzymes

INTRODUCTION

Throughout decades the chemical, biological, physical and social environments have been changed and the today's children living in a very different environment than from their parents and grandparents. Today's they are at most risk of exposing to new chemicals that are mostly not tested for fetus and children. Many of these chemicals can be accumulated in the mother's body before pregnancy and may cross over placental barrier during pregnancy. There is strong evidence that environmental pollutants increase oxidative stress and that dietary antioxidant supplementation may play a role in neutralizing the oxidizing effects of those pollutants (Poljšak and Fink, 2014). The possible connection between maternal toxic environmental exposures and increasing abortion rate, foetal
mortality and children morbidity is an emerging area of concern. Nowadays air pollution becomes a major environmental problem especially in developing countries like Egypt where the environmental issues not on the top of priorities due to other pressing economic problems (Arceo et al., 2016). Diesel exhaust particles (DEPs) considered the most abundant outdoor source of airborne particulate matter this may return to the widely using of diesel engines in transport, agricultural machinery, and power supply due to their low operating cost and significant power. A diesel engine produces about 100 times more particulate matter than a normal petrol engine; the particulate matter in diesel exhaust gas is a highly complex mixture of organic, inorganic, solid, volatile and partially volatile compounds. To date; few studies have considered particulate matter composition in relation to pregnancy outcomes (Michelle et al., 2010). DEPs exposure has been reported to have toxic effects by increasing oxidative stress and cell damage on various vital organs and systems, including respiratory system, immune system and nervous system. Organs like lung, heart, kidney, brain, skin, and liver are affected, resulting in various diseases and different forms of genotoxic, mutagenic, immunotoxic, and carcinogenic manifestations (Festus et al., 2013). Fenitrothion (FEN) is an organophosphorous insecticide used in Egypt for controlling a wide range of insects and pests (Fouad et al., 2008). Organophosphate insecticides have been shown to have toxic effects in humans (Abdel-Rasoul et al., 2008) and can cross the placental barrier and thus potentially affect the developing fetus (Jurewicz and Hanke, 2008). FEN has the potential to cause reproductive toxicity in animals (Abdel-Ghany et al., 2016) and affect human reproduction (Okamura et al., 2005). It was found that a degradation product of FEN, 3-methyl-4-nitrophenol (PNMC), could be extracted from diesel exhaust particles and have significant effects on human health via disruptions of reproductive and endocrine systems (Li et al., 2006). Flaxseed (Linum usitatissimum) is an Egyptian ancient plant grown especially for oil and fibers in different parts of the world (Kaithwas and Majumdar, 2013). However, flaxseed has a significant antioxidant potential, beside several nutritional values and health benefits, there is a lack of high quality researches on its effective uses. Several studies showed that flaxseed protect against various diseases like breast cancer, colon cancer, diabetes mellitus, atherosclerosis, and other diseases (Ivanov et al., 2011). Our study is believed to be the first study that will explores a new property of flaxseed for protection against toxicity of DEPs and/or FEN during pregnancy.

MATERIALS AND METHODS

Diesel Exhaust Particles Collection and Preparation

DEPs were collected after one day of routine operation of a microbus (Toyota 2008, 1300 cc diesel engine) from Helwan city’s Microbus Station. The particles collected using new soft toothbrush from the exhaust pipe then baked at 165 ºC for 3 hours to eliminate endotoxin, then stored on a dark glass bottle at 4 ºC for toxicological and analytical studies. For toxicological tests 1g of DEP was re-suspended in 100ml saline for 3hr. through magnetic stirring and was sonicated for 1hr using digital ultrasonic water bath. Next, the DEP was diluted to 300 µg of DEP in 100µl saline (Yoshizaki et al., 2015). The suspension was divided into 20 separate eppendorfs and stored at −80 ºC until use.

Fenitrothion

A technical grade (95% active ingredient) of the organophosphorous insecticide, Fenitrothion was purchased from Sumitomo Chemical Co. Ltd., (Tokyo-Japan).

Flaxseed

Fresh seeds were purchased from the local market in Egypt and authenticated by a botanist at botany department, Cairo University. The whole grain finely ground using electrical grinder. The diet (pellet concentrated diet) was also finely ground and divided into two parts one mixed with 15% ground flaxseed used for nutrition of the treated groups and the other part, without flaxseed, used as a diet of other groups.

Animals and Experimental Groups

The experimental work was done according to the guidance for care and use of laboratory animals and present study was approved by Ethics Committee for Institutional Animal Care and Use Committee (CU-IACUC) at Cairo University (CU/I/S 98/17). A total of 48 adult virgin females and 10 males weighing 200±10 grams and 13 to 14 weeks age were withdrawn from the breeding colony of the Mammalian Toxicology Dept., Central Agricultural Pesticides Lab, Giza, Egypt. The animals were randomly housed in...
appropriate plastic cages with stainless steel wire lids and wood shavings as bedding material. The cages were kept in air conditioned room at a temperature of 25±2 °C with a relative humidity of 50-60% and normal light/dark cycle. The animals were given the formulated study diet and tap water ad libitum. Three females had been transferred into each male cage early in the afternoon. Next morning timed pregnant females were then separated from male rats and housed in separate cages and classified into eight groups (6 dams for each group) as follow:

**Control group:**
Animals received an intranasal instillation of 100 µl saline solution (0.9% NaCl) 50 µl in each nostril and 100 µl oral gavage of corn oil and fed on flax free diet

**Flaxseed group (FS):**
Animals fed on diet mixed with 15 % flax.

**Diesel exhaust particles group (DEPs)**
Animals received an intranasal instillation of 100 µl DEPs suspension (1.5mg/Kg/day) 50 µl in each nostril and fed on flax free diet.

**Fenitrothion group (FEN)**
Animals received an oral gavage of 100 µl FEN (1/200 of LD50 = 3.76 mg/Kg) and ate flax free diet.

**DEPs+FEN group:**
Animals received an intranasal instillation of 100 µl DEPs suspension 50 µl in each nostril as well as an oral gavage of 100 µl FEN and fed on flax free diet.

**DEPs+FS group:**
Animals received an intranasal instillation of 100 µl DEPs suspension 50 µL in each nostril and fed on diet mixed with 15 % flax.

**FEN+FS group:**
Animals received an oral instillation of 100 µl FEN and fed on diet mixed with 15 % flax.

**DEPs+FEN+FS group:**
Animals received an intranasal instillation of 100 µl DEP suspension 50 µl in each nostril as well as an oral gavage of 100 µl FEN and fed on diet mixed with 15 % flax.

Flaxseed was added from 1st day of gestation while DEPs and FEN were administered at 6th day of gestation. The toxicity in the treated dams was determined by observing external symptoms, maternal mortality and changes in body weight. The daily record of their weights was made throughout the whole gestational period. The percent change in maternal body weight through the gestation was calculated by the following equation = (maternal wt. at 20th day - wt. at zero day/wt. at 20th day)x100 (Abdel-Rahman et al., 2017). The abortion rate and any morphological changed were monitored daily.

**Sampling and Biochemical Assays**
On the day 19th of gestation, the pregnant rats were slightly intraperitoneal anaesthetized with sodium pentobarbital 40mg/kg and the blood samples were collected in glass tubes from retro-orbital plexus according to the method of (Schalm, 1986). The tubes were immediately centrifuged using Sigma Laboratory Centrifuge (3K30, Germany) at 3600 r.p.m for 15 min. Serum samples were separated and kept at −80 °C until used for routine biochemical measurements. Then the animals were scarified and uteri were collected, implantation sites, number of fetuses and fetal mortality rate (resorbed or still birth) were recorded. Foetal body weight, length and any external abnormalities were recorded. Acetylcholinesterase enzyme (AchE) activity was determined according to Ellman et al., (1961) method with the modification proposed by Bisso et al., (1991). Lactate dehydrogenase (LDH) was determined using the kinetic ultraviolet method of (Vander et al., 1994). Total cholesterol (T. Chol.) was determined by Röschlau et al., (1974) method. Triglycerides were determined by Uwajima et al., (1984) method. High density lipoprotein–cholesterol level (HDL-C) was determined by Assmann (1979) method. The low density lipoprotein cholesterol (LDL-C) was calculated according Friedewald et al., (1972) equation. Urea was determined by the enzymatically colorimetric method of Bablok et al. (1988). Creatinine was measured according to Henry (1974) method. Serum glucose was determined by enzymatic colorimetric method of Trinder (1959).

**Statistical analysis**
A standard computer program SPSS for Windows, release 21.0 (IBM SPSS Inc, USA) was used for data entry and analysis. Mean values and standard error of the mean (mean ± SEM) were calculated for each tested group on the basis of values obtained from six individual rats. One-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD)
test for comparison between studies groups (Moore and McCabe, 2003). If \( p > 0.05 \), values were not statistically different while the \( ps < 0.05 \) level was set as statistically significant different.

**RESULTS AND DISCUSSION**

**Maternal clinical signs of toxicity**

During this experiment, no death was observed in any of the experimental groups. Intoxication with DEPs resulted in signs of toxicity on dams including general weakness, sneezing, coughing and nasal secretions, less food and water intake observed in all dams while nasal bleeding observed in about 50% of intoxicated dams. While oral gavage of FEN resulted in salivation, lacrimation, flat body appearance, sweating, and diarrhea. These signs became highly significant on the combined group that received both DEPs and FEN, the signs were appeared on the fifth and third day of treatment of DEPs and FEN respectively and progressed in the same animals throughout the period of the treatment. No symptoms of toxicity were observed in the dams fed on flaxseed until the end of the experiment. The toxic signs produced by DEPs and/or FEN were indicative of nervous system involvement may be due to rapid and complete absorption of these compounds which may be result in accumulation of the parent compounds and/or its metabolites following direct administration.

**Protective role of flaxseed against the toxic effects of DEPs and/or FEN on some maternal biochemical parameters.**

**Acetylcholinesterase activity (µM/min/ml).**

Intoxication of the pregnant rats with DEPs and/or FEN induced a significant \( (p < 0.05) \) reduction in AchE activities as compared with normal control. Supplementation with 15% Flaxseed significantly \( (p < 0.05) \) increased AchE activity in all treated groups as compared with their parallel intoxicated ones (Table 1). The high significant \( (p < 0.001) \) inhibition of AchE activities in FEN and FEN+DEPs groups as compared with DEPs group may resulting from oxone formation after FEN microsomal oxidation in the liver, which is a potent inhibitor of AchE in the peripheral blood and tissues. The diminish in AchE activity due to DEPs intoxication may be due to the presence of polycyclic hydrocarbon and/or heavy metals that increase oxidative stress and free radicals in the brain leading to inhibition of major brain neurotransmitters (Win-Shwe et al., 2015). Improving AchE activity in FS-supplemented groups may be due to the presence of omega-3 polyunsaturated fatty acids and potent antioxidants compounds that decrease oxidative stress in the brain and thus improving brain neurotransmitters, moreover omega-3 polyunsaturated fatty acids, from supplementation, may be replaced with polyunsaturated fatty acids components of the membranes that had been attacked by oxygen free radicals (Badawy et al., 2015).

Lactate dehydrogenase activity (µM/min/ml). Concerning the toxic effect of DEPs and/or FEN on maternal lactate dehydrogenase (LDH) enzyme, our study revealed a significant \( (p < 0.05) \) increase on maternal serum LDH activity after intoxication with DEPs and/or FEN. A marked decrease \( (p < 0.05) \) in LDH activity was observed in all FS- supplemented groups as compared with their parallel intoxicated ones (Table1). DEP and/or FEN may increase oxidative stress that may lead to myocardial injury and tissue damage which may lead to leakage of LDH enzyme to the cytoplasm of myocardial cell and then to blood circulation, the strong antioxidant activity of FS may decrease this oxidative stress and protect the myocardial tissues from injury and damage since no more LDH released. Previous studies correlated increased levels of LDH with exposure to particulate air pollution (Gurgueira et al., 2002 and Dong et al., 2005). Little data is present about the effect of FEN on heart, however Anand et al., (2009) showed that potentially lethal cardiac complications occur in patients with acute organophosphate poisoning these complications injured myocardial cells and lead to leakage on cardiac LDH.

**Lipid profile**

With regard to lipid profile a significant \( (p < 0.05) \) increase in maternal serum total cholesterol, triglycerides and LDL but a decrease in HDL as a result of intoxication with DEPs and/or FEN. Diminished levels of cholesterol, triglycerides and LDL while elevated levels of HDL were observed in all FS treated-pregnant as compared with parallel intoxicated ones (Table 2). Our results go in the line with Ticknor (2014) who found that diesel exhaust increase triglycerides and total cholesterol, but more specifically, higher values of VLDL and LDL and lower values of HDL in pregnant mice beside that the majority of altered genes within “lipid metabolism” were also down-regulated after diesel exhaust exposure.
Table (1): Effect of intoxication with DEPs and/or FEN and protective role of flaxseed supplementation on maternal serum acetylcholinesterase and lactate dehydrogenase activities (μM/min/ml).

<table>
<thead>
<tr>
<th>Groups</th>
<th>AChE activity</th>
<th>LDH activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1198.74 ± 3.37</td>
<td>207 ± 2.95</td>
</tr>
<tr>
<td>FS</td>
<td>1189 ± 2.88</td>
<td>196.52 ± 1.77</td>
</tr>
<tr>
<td>DEPs</td>
<td>941 ± 3.24a,b,d,e</td>
<td>322.4 ± 3.23a,b,d,e</td>
</tr>
<tr>
<td>FEN</td>
<td>412.11 ± 5.14a,b,e</td>
<td>520.54 ± 3.33a,b,e</td>
</tr>
<tr>
<td>DEPs+FEN</td>
<td>342.65 ± 4.36a,b</td>
<td>569.23 ± 3.63a,b</td>
</tr>
<tr>
<td>DEPs+FS</td>
<td>1001.7 ± 2.28c,g,h</td>
<td>216.94 ± 1.89c,g,h</td>
</tr>
<tr>
<td>FEN+FS</td>
<td>701 ± 2.96d,h</td>
<td>318.88 ± 2.69d,h</td>
</tr>
<tr>
<td>DEP+FEN+FS</td>
<td>498.29 ± 3.24e</td>
<td>413.78 ± 4.66e</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE. One-way analysis of variance (ANOVA) Turkey’s honestly significant difference (HSD) test was used, the mean difference is non-significant at P Value > 0.05 while the p ≤ 0.05 level was set as statistically significant different. (a); Significant compared to Control, (b) significant compared to FS, (c); significant compared to DEPs (d); significant compared to FEN, (e); significant compared to DEPs+FEN(f); significant compared to DEPs+FS(g); significant compared to FEN+FS, (h); significant compared to DEPs+FEN+FS [FS: Flaxseed; DEPs; Diesel exhaust particles; FEN; Fenitrothion].

Table (2): Effect of intoxication with DEPs and/or FEN and protective role of flaxseed supplementation on maternal serum total cholesterol, triglycerides, low density lipoprotein-cholesterol and high density lipoprotein-cholesterol concentrations (mg/dl).

<table>
<thead>
<tr>
<th>Items</th>
<th>T. Chol.</th>
<th>TG</th>
<th>LDL-C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92.5 ± 0.93</td>
<td>81 ± 0.44</td>
<td>28.19 ±1.23</td>
<td>48.12 ± 0.62</td>
</tr>
<tr>
<td>FS</td>
<td>81.3 ± 0.60a</td>
<td>72.63 ± 1.08a</td>
<td>14.36 ± 1.32a</td>
<td>52.42 ± 1.08a</td>
</tr>
<tr>
<td>DEPs</td>
<td>131.12 ± 1.39a,b,d,e</td>
<td>109.13 ± 2.3a,b,d,e</td>
<td>78 ± 1.36a,b,d,e</td>
<td>31.28 ± 1.68a,b,e</td>
</tr>
<tr>
<td>FEN</td>
<td>121.18 ± 1.71a,b,e</td>
<td>98.96 ± 1.49a,b,e</td>
<td>69 ± 1.78a,b,e</td>
<td>32.36 ± 0.97a,b,e</td>
</tr>
<tr>
<td>DEPs+FEN</td>
<td>148 ± 0.72a,b</td>
<td>138.91 ± 2.64a,b</td>
<td>95.61 ± 1.7a,b</td>
<td>24.69 ± 0.74a,b</td>
</tr>
<tr>
<td>DEPs+FS</td>
<td>100.59 ± 0.49c</td>
<td>94.9±1.44c</td>
<td>38.96 ± 1.47c,h</td>
<td>42.64 ± 1.79c,h</td>
</tr>
<tr>
<td>FEN+FS</td>
<td>99.7 ± 0.75d</td>
<td>90.35±0.33d</td>
<td>39.97 ±2.76d,h</td>
<td>41.66 ± 2.24d</td>
</tr>
<tr>
<td>DEP+FEN+FS</td>
<td>103.67 ± 1.19e</td>
<td>96.84±1.98e</td>
<td>48.59 ± 0.87e</td>
<td>35.7 ± 1.39e</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE. One-way analysis of variance (ANOVA) Turkey’s honestly significant difference (HSD) test was used, the mean difference is non-significant at P Value > 0.05 while the p ≤ 0.05 level was set as statistically significant different. (a); Significant compared to Control, (b) significant compared to FS, (c); significant compared to DEPs (d); significant compared to FEN, (e); significant compared to DEPs+FEN(f); significant compared to DEPs+FS(g); significant compared to FEN+FS, (h); significant compared to DEPs+FEN+FS [FS: Flaxseed; DEPs; Diesel exhaust particles; FEN; Fenitrothion].

Such results support the hypothesis that in utero to diesel exhaust exposure created an environment that increased susceptibility to cardiovascular disease long term. Disturbances in lipid profile due to intoxication with FEN may be due to its effect on hepatocyte cell membrane permeability or blockage of bile duct that stop or reduce cholesterol secretion to duodenum. Also, this may be due to reduced hepatic lipase activity that affect lipid metabolism (Kalender et al., 2010). The hypolipidemic effects of whole flaxseed may be due to lignans content and soluble fibers that modulate activities of 7-hydroxylase and acyl CoA cholesterol transferase, two of the key enzymes involved in cholesterol metabolism. Omega-3 fatty acid can decrease TG by decreasing hepatic lipogenesis (Cornish et al., 2009).

Kidney function tests serum levels (urea and creatinine)
Our study showed that intoxication with DEPs or FEN caused a slight non- significant (p>0.05) elevation in maternal serum urea as compared with control but their combination (DEPs+FEN) caused a significant (p<0.05) increase as compared with control. DEPs was able to affect the creatinine level non-significantly (p>0.05) as
compared with control, while FEN alone or in combination with DEPs significantly (p<0.05) increased creatinine level as compared with normal pregnant rats. The consumption of FS was able to ameliorate kidney functions of DEPs and/or FEN intoxicated pregnant rats by decreasing urea and creatinine concentrations in all FS supplemented groups as compared with their parallel intoxicated ones (Table 3). Kidney consider one of target organs of organophosphates (Abdel-Reheim, 2008), increasing urea and creatinine due to FEN intoxication signalizing kidney functional damage that increased especially in the combined group (DEPs+FEN). The oxidative stress cause by DEPs may cause kidney cells injury (Al-Suleimani et al., 2016) and decreasing oxidative stress by antioxidant activity of FS may decrease kidney injury and damage.

**Serum concentration of glucose**

In this study, a significant (p<0.05) increase in glucose level was observed due to intoxication pregnant rats with DEPs and/or FEN compared with normal control rats. In contrast, FS supplementation reduced glucose levels in all treated groups when compared with their parallel intoxicated ones (Table 3). Increase glucose level after intoxication with DEPs may be due to insulin resistance as the high lipid resulting from exposure to such toxic substances or inflammation of pancreas by resulting oxidative stress (Shen et al., 2017 and Wilson et al., 2018). High glucose levels that observed in FEN intoxicated group might be due to disturbances in glucose transport and/or glycogen metabolism. Moreover, an imbalance between hepatic output of glucose and its peripheral uptake can increase glucose level or as a consequence of suddenly increased catabolism to encounter higher organophosphate stimulated energy demands (Abdel-Ghany et al., 2016). FS bioactive component’s especially omega-3 polyunsaturated fatty acids may increase insulin sensitivity and reducing oxidative stress in pancreas (Pacheco et al., 2011).

**Protective role of FS against in utero exposure of pregnant Albino rats to DEPs and/or FEN**

As shown in Table (4), intoxication pregnant rat with DEPs and/or FEN from day 6-19 of gestation caused a significant (p<0.05) reduction in maternal body weight gain and uterus weight as compared with control and FEN beside DEPs+FEN groups scored a higher reduction than DEPs group. Also, embryonic growth retardation was observed in DEPs and/or FEN groups that represented by a significant reduction (p<0.05) in placental weight, foetal weight, foetal length, increased resorption and decreased foetuses number were also observed in these groups as compared with control. Furthermore, intoxication with DEPS and/or FEN caused many external abnormalities such as deformed limbs, hematoma, herniation and also tumor. However; FS-treated groups showed increased %change in maternal body weight as well as uterus weight as compared with their parallel intoxicated ones. Moreover, FS was able to protect foetus health from intoxication by DEPs and/or FEN by increasing foetus weight, length and placental weight in all FS treated groups as compared with their parallel intoxicated ones.

Table (3): Effect of intoxication with DEPs and/or FEN and protective role of flaxseed supplementation on maternal serum levels of kidney function tests, urea and creatinine (mg/dl) and glucose concentrations (mg/dl).

<table>
<thead>
<tr>
<th>Items</th>
<th>Urea</th>
<th>Creatinine</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47.63 ± 2.27</td>
<td>1.05 ± 0.02</td>
<td>127.50 ± 0.73</td>
</tr>
<tr>
<td>FS</td>
<td>49.56 ± 0.92</td>
<td>0.95 ± 0.043</td>
<td>120.6 ± 0.45</td>
</tr>
<tr>
<td>DEPs</td>
<td>49.84 ± 1.24e</td>
<td>1.22 ± 0.055b</td>
<td>137.4 ± 0.93a,b,d</td>
</tr>
<tr>
<td>FEN</td>
<td>49.69 ± 1.69e</td>
<td>1.33 ± 0.10a,b</td>
<td>131.9 ± 1.14a,b,e</td>
</tr>
<tr>
<td>DEPs+FEN</td>
<td>64.46 ± 2.49a,b</td>
<td>1.37 ± 0.056a,b</td>
<td>141.6 ± 0.93a,b</td>
</tr>
<tr>
<td>DEPs+FS</td>
<td>44.59 ± 1.11</td>
<td>1.02 ± 0.039</td>
<td>123.09 ± 0.83c,g,h</td>
</tr>
<tr>
<td>FEN+FS</td>
<td>46.16 ± 2.79</td>
<td>0.97 ± 0.021d</td>
<td>116.1 ± 1.49d,h</td>
</tr>
<tr>
<td>DEP+FEN+FS</td>
<td>48.07 ± 0.92e</td>
<td>1.058 ± 0.036e</td>
<td>133.10 ± 0.69f,e</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE. One-way analysis of variance (ANOVA) Turkey’s honestly significant difference (HSD) test was used, the mean difference is non-significant at P Value > 0.05 while the p ≤ 0.05 level was set as statistically significant different. (a); Significant compared to Control, (b) significant compared to FS, (c); significant compared to DEPs (d); significant compared to FEN. (e); significant compared to DEPs+FEN(f); significant compared to DEPs+FEN+FS(g); significant compared to FEN+FS, (h); significant compared to DEPs+FEN+FS [FS: Flaxseed; DEPs; Diesel exhaust particles; FEN; Fenitrothion].
Table (4): Pregnancy outcomes in different studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cont.</th>
<th>FS</th>
<th>DEPs</th>
<th>FEN</th>
<th>DEPs+ FEN</th>
<th>DEPs+ FS</th>
<th>FEN + FS</th>
<th>DEPs+ FEN+FS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dams No.</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Pregnancy (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Change of maternal Weight (%)</td>
<td>35.18±0.33</td>
<td>24.64±0.45a</td>
<td>12.05±0.68a,b,d,e</td>
<td>6.66±0.49a,b</td>
<td>6.42±0.48a,b</td>
<td>17.88±0.49c,g</td>
<td>12.022±0.58d,h</td>
<td>15.80±0.46e</td>
</tr>
<tr>
<td>Weight of gravid uterus</td>
<td>68.67±0.99</td>
<td>68±0.73</td>
<td>32.83±4.6a,b</td>
<td>26.33±0.84a,b</td>
<td>23.67±2.67a,b</td>
<td>52.17±1.9c</td>
<td>43.33±1.12d,h</td>
<td>54.83±2.12e</td>
</tr>
<tr>
<td>Number of implants</td>
<td>10.5±0.34</td>
<td>10.5±0.22</td>
<td>8.8±0.65</td>
<td>7.5±0.22a,b</td>
<td>8.67±0.33a,b</td>
<td>9.67±0.33</td>
<td>9.67±0.42d</td>
<td>10.12±0.48</td>
</tr>
<tr>
<td>Resorbed Fetuses (%)</td>
<td>0</td>
<td>0</td>
<td>21</td>
<td>11</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Weight of placenta (mg)</td>
<td>926.8±10.04</td>
<td>994.8±25.10</td>
<td>441.67±34.36a,b</td>
<td>428.57±48.79a,b</td>
<td>305.32±48.166a,b</td>
<td>550.46±43.69c,h</td>
<td>587.44±29.97d</td>
<td>458.83±18.99</td>
</tr>
<tr>
<td>Foetal weights (g)</td>
<td>5.47±0.74</td>
<td>5.16±0.37a</td>
<td>2.4390±0.55a,b,e</td>
<td>2.1025±0.55a,b</td>
<td>1.7892±0.46a,b</td>
<td>3.6034±0.49c,g</td>
<td>2.5776±0.44d,h</td>
<td>3.5263±0.50e</td>
</tr>
<tr>
<td>Foetal length</td>
<td>5.18±0.079</td>
<td>5.40±0.13</td>
<td>3.17±0.042a,b,e</td>
<td>2.9±0.037a,b</td>
<td>2.6±0.058a,b</td>
<td>4.43±0.061c,g,h</td>
<td>4.02±0.031d</td>
<td>4±0.086e</td>
</tr>
<tr>
<td>Hematoma (%)</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>17</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Oligodactyl (%)</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Herniation (%)</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>11</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE. One-way analysis of variance (ANOVA) Turkey's honestly significant difference (HSD) test was used, the mean difference is non-significant at P Value > 0.05 while the p ≤ 0.05 level was set as statistically significant different. (a); Significant compared to Control, (b) significant compared to FS, (c); significant compared to DEPs (d); significant compared to FEN, (e); significant compared to DEPs+FEN, (f); significant compared to DEPs+FS, (g); significant compared to FEN+FS, (h); significant compared to DEPs+FEN+FS [FS: Flaxseed; DEPs; Diesel exhaust particles; FEN; Fenitrothion].

Bioscience Research, 2018 volume 15(3): 1832-1844
Figure (1): photographs showing lateral view of foetus’s size obtained at 19th day of gestation in Control (A), FS (B), intoxicated groups [DEPs (C), FEN (D), &DEPs+FEN (E)] and treated groups [DEPs+FS (F), FEN+FS (G) &DEPs+FEN+FS (H)]: the photos observe the difference in sizes between Control and intoxicated groups. Addition of flaxseed in pregnant rat’s diet increase the foetuses size and their shape shifted to normal as shown in the above figure in the treated groups. [FS: Flaxseed; DEPs; Diesel exhaust particles; FEN; Fenitrothion].

Figure (2): Some photographs show the resorbed foetuses (arrows) due intoxication with DEPs and/or FEN (A: DEPs, B: FEN and C DEPs+FEN). [DEPs; Diesel exhaust particles; FEN; Fenitrothion].
Furthermore, about 90% of external abnormalities and 100% of resorption was disappeared in FS-treated groups Figures (1&2). The uteri of normal control pregnant rats on gestational day 20th had a normal distribution of implanted foetuses between the two horns. The uteri of the intoxicated pregnant rats showed asymmetrical distribution of foetuses in the two uteri tubes, reduced number of fetuses and embryonic resorptions (Figure 3). The reduction in maternal weight in the current study may be attributed to starvation or malnutrition which caused by DEPs and/or FEN or foetal resorption and foetal growth retardation.

Figure (3): Some morphological abnormalities (arrows) observed in foetuses due to intoxication their mothers with DEPs and/or FEN from 6th day to 19th day of gestation. Intoxication with DEPs caused (A: Herniation, B: Hematoma at back head, C: Hematoma at neck and tail, E: Loss of hand digits), intoxication with FEN caused (F: hematoma at back, G: Hematoma at tail, herniation at abdomen & neck H: Herination at abdomen end). Intoxication with DEPs+FEN: All photos showed the small foetuses size and most body features were undefined (I: hematoma, loss hand digits and herniation. J: loss hand digits, herniation and forelimb, K: tumor at upper part of head & hematoma. L: hematoma).
CONCLUSION
Daily uses of flaxseed are important especially in vegetarian females as a good source of omega-3 fatty acids and lignans but should be not exceeding 5% (1-2 teaspoons/day).

CONFLICT OF INTEREST
No conflict of interest in this study.

ACKNOWLEDGEMENT
The author would thank all participants in this work and their families.

AUTHOR CONTRIBUTIONS
In this study all authors contributed equally

REFERENCES


Flaxseed alleviates pollution toxicity on pregnant rats and their foetuses


