Corresponding Author: Nashwah I. Zaki, Departments of Physiology, National Organization for Drug Control and Research (NODCAR), Egypt.
E-mail:nashwahizaki@yahoo.com

ABSTRACT

The current investigation aimed to evaluate endocrine disruption and oxidative stress of subchronic co-exposure of Malathion (500 ppm), Arsenic (50 ppm) and Paracetamol (100 mg/kg b.w) in white rats. Eighty adult male albino rats weighing 120-140 g were orally treated with these agents individually or in-combination for 28 days (4weeks). Changes in body weight gain, blood total triiodothyronin (T3), thyroxin (T4), testosterone, lipid peroxidation (MDA), total antioxidants capacity (TAC) and total proteins (TP); besides some histopathological and morphometrical analysis of testis and thyroid tissues were evaluated. Exposure to selected xenobiotics individually or combined showed general decrease in body weight; a significant reduction in T3 level in Paracetamol combined treated groups; a significant increase in T4 among Arsenic treated groups; as well as reduction in testosterone level in all treated groups. A significant elevation in MDA concomitant with reduction in TAC and significant increase in TP level were recorded in all treated groups as compared to control level. Histological examination revealed spermatogenic arrest, edema in interstitial spaces in most treated groups, meanwhile thyroid tissues displayed cystic dilatation in thyroid follicles, colloidal depletion and degenerative changes in folliculocytes. Morphometric measurements showed reduction in Leydig cells count, reduction in follicular cells height reflecting their impairments which confirm the biochemical changes. Join action analysis of these xenobiotics reflected synergistic reactions on body weight and testosterone activity, whereas appeared to be antagonistically on T3, T4 and TAC. On the other hand, MDA and total protein showed additive effects. These results revealed that Malathion, Arsenic and Paracetamol reacted differentially to the tested parameters.

Key words: Endocrine disruption, organophosphate, metalloid, analgesic drugs, histopathology and morphometric analysis, testis, thyroid gland.

Introduction

A growing body of evidence suggests that natural and man-made chemicals may interfere with the endocrine system and produce adverse effects in laboratory animals and humans. Scientists often refer to these chemicals as EDC's "endocrine disruptors". The Natural Resources Defense Council (NRDC, 1998) defined endocrine disrupters as synthetic chemicals that when absorbed into the body either mimic or block hormones and disrupt the body's normal functions by halting or stimulating hormones production, or by changing their mode of action. In that concern, their potential adverse effects on human health arises from the fact that endocrine- disrupting chemicals although present in the environment at very low levels, yet they exert adverse effects in wildlife species as well as in laboratory animals. Furthermore, the effects of different classes of EDCs may be additive or even synergistic (Crews et al., 2003). Generally, exposure to mixture of chemicals induces alteration in biological responses (Institoris et al., 2001; Wilkinson, 2001). Therefore, endocrine assessment is a useful diagnostic tool in the detection of early or low-level responses to pollutants that may precede more significant health problems (Kendall et al., 1998). Most of the studies on endocrine disruption or modulation have been focused on reproductive problems and behavioral abnormalities related to reproduction, although contaminants may target other parts of the endocrine system (Damstra et al., 2002; Hinson and Raven 2006). The hypothalamus–pituitary adrenal (HPA) axis is an important system that regulates and integrates many physiologic functions in response to environmental stressors (Wingfield and Kitaysky 2002). Thyroid hormones - thyroxin (T4) and triiodothyronine (T3) play an important role in metabolism as well as mammals 'maturity (Jaff and Salih, 2009). Additionally, thyroid hormones control the body's entire oxidant/antioxidant system and can influence the activity of SOD and CAT in liver via a thyroid independent pathway (Bolzan et al., 1995).

Among the pollutants available in Egypt, Malathion conforms one of the most widely used pesticides in agriculture and public health practices worldwide and Arsenic is an environmental contaminant. Meanwhile,
Paracetamol is an extensively used non-steroidal analgesic and antipyretic drug. The interaction between the environmental pollutants Arsenic and/or Malathion is of great applied significance as humans exposed to these chemicals which may concurrent with the treatment with Paracetamol for relieving pains. Despite these facts, little is known about their deleterious endocrine disruptor effects. Thereby; the present study was designed to determine the endocrine disruptor; toxicity and oxidative stress effects, of low level subchronic exposure of Malathion, Arsenic, Paracetamol and their combination in rats.

Materials and Methods

Chemicals:

Malathion 57% CE emulsion concentrates (Awida-Egypt) was purchased from local market. Dose of Malathion (500 ppm) was based on previous studies (Akhgari et al., 2003; Abdollahi et al., 2004). Sodium Arsenite: (NaAsO2) was obtained from Sigma Chemical Co (St. Louis). Arsenic (50 ppm) was within the range of LD50 of a 70-Kg body wt. human (1-4 mg/Kg) and lesser than 1/25 of LD50 of rats (40mg/kg) according to Mukherjee et al. (2003; 2004). Paracetamol tablets were purchased from local pharmacy; each tablet contains 500 mg Paracetamol. The therapeutic dose for rat was (100 mg/kg) a non-toxic dose according to Naziroğlu et al. (2009).

Experimental Design:

Eighty adult male albino rats weighing 120-140 g were supplied by National Organization for Drug Control and Research animal house. They were housed in wire cages with natural ventilation and illumination and allowed free water and standard diet for 10 days before beginning of the experiment, and then they were randomly assigned into eight main groups, ten rats each. Group 1 served as control. Groups 2, 3 and 4 were given individual treatment of Malathion (M; 500 ppm), Arsenic (A; 50 ppm) or Paracetamol (P; 100 mg/kg b.w), respectively. Groups 5, 6 and 7 rats were given paired treatments as follow G5 (M+A), G6 (M+P) and G7 (A+P); while rats of G 8 were given combined treatment (M+A+P). All rats received the doses as daily oral treatments dissolved in distilled water via a gastric tube for 28 days. At the end of the experimental period, blood samples were collected from retro-orbital plexus vein, allowed to clot for thirty minutes at room temperature and centrifuged at 1000 x g and 4ºC for ten minutes. The collected serum samples were stored at -20 ºC for estimation of hormone levels and other biochemical parameters. All procedures and the experimental protocols were approved by the Institutional Ethics Committee at NODCAR and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals. Initial body weight and external symptoms were weekly recorded through the experimental period. Final body weight of control and treated rats were measured at the end of the 4th week.

Hormonal and Biochemical Analysis:

Testosterone, triiodothyronin and thyroxin levels were measured by using a chemiluminescence immunoassay-based commercial kit according to Witherspoon et al., 1988. Malondaldehyde level (Lipid peroxidation; LPO) was measured by estimation of thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa et al., 1979. Spectrophotometric procedure was used for the determination of total antioxidants capacity in serum using commercial kit according to Koracevic et al., 2001. Standard and quantitative assay for protein content was evaluated in serum by the method of Bradford, (1976).

Histopathological Analysis:

The studied organs were removed and weighed immediately after anesthesia. Small pieces of testis and thyroid tissues were fixed in 10 % formalin solution, and then proceeded until embedding in paraffin. Serial sections were prepared at 4μ, then, stained with haematoyxlin and eosin (H&E) (Banchroft et al., 1996).The sections were examined and photographed by using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached photograph camera (Olympus E-330, Olympus Optical Co. Ltd., Japan).

Morphometrical Measurements:

Number of Leydig Cells:

Ten random fields of testis tissue per animal were examined in H&E stained slides using (100X) power and counted the number of Leydig cells according to Otoom et al. (2004).
Thyroid Follicular Diameter and Follicular Epithelium Height:

Image analysis system was used to determine the diameter and epithelium height of thyroid follicles (Image Proplus version 5). The internal follicular diameter was measured in 50 follicles in ten random fields of the thyroid gland of each rat using low power (4X). They were measured on diagonal axes and the mean of these two readings was taken as the internal diameter of each follicle. Epithelium height was measured in 50 follicles per rat using (40X) power at two points on the same axis of each follicle, the mean of these two readings was taken as the epithelium height according to Kalisnik, (1981).

Statistical Analysis:

The data obtained from the biochemical analysis of different groups are represented in tables as Mean ± Standard Error (mean ± SE). The significance of the difference between the groups was calculated by one-way analysis of variance (ANOVA) followed by Tukey’s procedure for multiple comparisons at P < 0.05 using the SPSS-PC computer software package version 17. However, joint action analysis was calculated using “relative interaction index” (RII) according to Mansour et al. (2001).

Results:

Percentage of weekly body weight gain of the rats received different treatments was depicted in (Table 1). Data indicated that, rats in control group achieved 7.48% weekly body weight gain. Generally, different treatments achieved less body weight gain as compared with the control one, individual treatments of Malathion, Arsenic and Paracetamol induced weekly body weight gain less than the control one by 1.92%, 2.50% and 1.88% respectively. In combined treatments, weekly body weight gain were severely affected in rats treated with M+P (-1.59%) and M+A+P (0.37%) and the least effective treatment was A+P (3.40%).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial body weight (g) ± SE</th>
<th>Final body weight (g) ± SE</th>
<th>% of weekly body weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>123.00±2.92</td>
<td>159.80±1.93</td>
<td>7.48%</td>
</tr>
<tr>
<td>M</td>
<td>140.40±4.50</td>
<td>151.20±1.79</td>
<td>1.92%</td>
</tr>
<tr>
<td>A</td>
<td>139.80±5.10</td>
<td>153.80±2.28</td>
<td>2.50%</td>
</tr>
<tr>
<td>P</td>
<td>140.80±5.68</td>
<td>151.40±2.36</td>
<td>1.88%</td>
</tr>
<tr>
<td>M+A</td>
<td>128.60±4.22</td>
<td>140.50±2.45</td>
<td>2.31%</td>
</tr>
<tr>
<td>M+P</td>
<td>131.40±2.67</td>
<td>123.00±2.82</td>
<td>-1.59%</td>
</tr>
<tr>
<td>A+P</td>
<td>120.60±2.93</td>
<td>137.00±1.88</td>
<td>3.40%</td>
</tr>
<tr>
<td>M+A+P</td>
<td>140.20±4.97</td>
<td>142.25±4.07</td>
<td>0.37%</td>
</tr>
</tbody>
</table>

Relative Interaction Index (R.I.I.)

-% of weekly body weight gain= Mean final body weight-Mean initial body weight/Mean initial body weight X no. of weeks.
-Results were expressed as mean ± SE for each 7 rats.

Depending upon the hypothetical method posed in Mansour et al. (2001) study, the data given in (Table 1) were used to estimate the suggested "Relative Interaction Indices (RII)" for the tested treatments as shown in (Table 2) mixture treated groups showed the following indices: M+A (1.05); M+P (-0.84); A+P (1.55) and M+A+P (0.17). This means that the mixture groups M+A and A+P were interacted antagonistically. While, potentiating effects were entitled to M+P and M+A+P treatments.

As constructed in (Table3) significant decrease in T3 level were entitled to combined treatments (M + P; -36.34%), (A + P; - 45.79%) and (M + A + P; -37.89%) as compared to control. In contrast, significant increase in T4 level among individual treatments with MA; As and binary treatment (A + P) when compared to control level (15.06±0.66 nmol/L; P<0.05). Whereas, remarkable significant decrease was recorded in testosterone level among all the groups under investigation when compared to control at P<0.05; reached its minimal level in binary treatment Arsenic plus Paracetamol (A + P) by -58.49% to that found in control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mixture value (%)</th>
<th>Av. value of individual compounds (%)</th>
<th>Relative Interaction Index (RII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+A</td>
<td>2.31</td>
<td>2.21</td>
<td>1.05 (An)</td>
</tr>
<tr>
<td>M+P</td>
<td>-1.60</td>
<td>1.90</td>
<td>-0.84 (Po)</td>
</tr>
<tr>
<td>A+P</td>
<td>3.40</td>
<td>2.19</td>
<td>1.53 (An)</td>
</tr>
<tr>
<td>M+A+P</td>
<td>0.37</td>
<td>2.10</td>
<td>0.17 (Po)</td>
</tr>
</tbody>
</table>

-Relative Interaction Index (R.I.I.)<1: potentiating or synergistic=Sy
-Relative Interaction Index (R.I.I.)>1: Antagonistic=An
-Relative Interaction Index (R.I.I.)=1: Additive i.e no observed effect=Ad
-According to Mansour et al. (2001) for body weight gain
Table 3: Hormonal profile of adult white male rats exposed to different treatments for four weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T3 nmol/L</th>
<th>T4 nmol/L</th>
<th>Testosterone nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.9±0.12</td>
<td>15.06±0.66</td>
<td>12.6±0.15</td>
</tr>
<tr>
<td>M</td>
<td>1.48±0.03</td>
<td>35.29±2.14</td>
<td>7.38±0.07</td>
</tr>
<tr>
<td>A</td>
<td>1.89±0.16</td>
<td>65.14±3.99</td>
<td>7.98±0.18</td>
</tr>
<tr>
<td>P</td>
<td>1.88±0.08</td>
<td>19.17±1.18</td>
<td>6.71±0.08</td>
</tr>
<tr>
<td>M+A</td>
<td>1.57±0.09</td>
<td>17.18±1.12</td>
<td>7.12±0.11</td>
</tr>
<tr>
<td>M+P</td>
<td>1.21*±0.08</td>
<td>21.38*±0.69</td>
<td>10.6*±0.10</td>
</tr>
<tr>
<td>A+P</td>
<td>1.03*±0.04</td>
<td>51.54*±3.28</td>
<td>5.23*±0.14</td>
</tr>
<tr>
<td>M+A+P</td>
<td>1.18*±0.10</td>
<td>21.21*±0.32</td>
<td>9.97*±0.15</td>
</tr>
</tbody>
</table>

-Results were expressed as mean ± SE for each 7 rats.

*Significance difference versus control at P < 0.05.

-a, b, c Significance difference of the mixtures versus M, A, P; respectively at P < 0.05.

Table 4: Oxidative stress profile of adult white male rats exposed to different treatments for four weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA mmol/100 ml</th>
<th>TAC mmol/L</th>
<th>TP g/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.58±0.13</td>
<td>1.26*±0.013</td>
<td>6.09±0.15</td>
</tr>
<tr>
<td>M</td>
<td>13.28±0.76</td>
<td>0.61*±0.054</td>
<td>6.95±0.51</td>
</tr>
<tr>
<td>A</td>
<td>14.07±0.76</td>
<td>0.34*±0.026</td>
<td>9.06*±0.40</td>
</tr>
<tr>
<td>P</td>
<td>7.65±0.14</td>
<td>0.25*±0.025</td>
<td>7.51±0.20</td>
</tr>
<tr>
<td>M+A</td>
<td>6.99*±0.2</td>
<td>0.77*±0.009</td>
<td>6.42±0.32</td>
</tr>
<tr>
<td>M+P</td>
<td>10.84*±0.44</td>
<td>0.28*±0.026</td>
<td>8.51*±0.44</td>
</tr>
<tr>
<td>A+P</td>
<td>7.15*±0.22</td>
<td>1.07*±0.007</td>
<td>8.95*±0.33</td>
</tr>
<tr>
<td>M+A+P</td>
<td>11.78±0.73</td>
<td>0.17*±0.017</td>
<td>8.07±0.48</td>
</tr>
</tbody>
</table>

-Results were expressed as mean ± SE for each 7 rats.

*Significance difference versus control at P < 0.05.

-a, b, c Significance difference of the mixture versus M, A, P; respectively at P < 0.05.

Table 4 showed pronounced elevation in MDA level lipid peroxidation biomarker concurrent with significant decrease in total antioxidant content (TAC) in all tested groups causing the minimal percentage change in combined treatment (M+A+P; -86.51%) compared to control at P < 0.05. Concerning the total protein content, general rise in its level was recorded among all animals reaching significant levels in rats exposed to Arsenic alone and Paracetamol combined treatment groups with a percentage change from control of 58.62%, 39.73%, 46.96% and 32.51% respectively at the end of the experimental period.

Regarding the relative interaction indices for the tested parameters, the tested combined treatment with (M+A) showed an obvious antagonistic effect to the tested parameter. (M+P) and (A+P) co-exposure showed an equilibrium between synergistic and antagonistic effect on biochemical parameters table 5.

Table 5: Joint action analysis for different treatments based upon final biochemical results in the treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters</th>
<th>T4</th>
<th>T3</th>
<th>Testosterone</th>
<th>MDA</th>
<th>TAC</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+A</td>
<td>0.93An</td>
<td>0.342 An</td>
<td>0.93 An</td>
<td>0.55 An</td>
<td>1.04 Sy</td>
<td>0.77 An</td>
<td></td>
</tr>
<tr>
<td>M+P</td>
<td>0.72 An</td>
<td>0.79 An</td>
<td>1.50 Sy</td>
<td>1.04 Sy</td>
<td>0.51 An</td>
<td>1.17 Sy</td>
<td></td>
</tr>
<tr>
<td>A+P</td>
<td>0.55 An</td>
<td>1.22 Sy</td>
<td>0.71 An</td>
<td>0.66 An</td>
<td>3.63 Sy</td>
<td>1.04 Sy</td>
<td></td>
</tr>
<tr>
<td>M+A+P</td>
<td>0.67 An</td>
<td>0.53 An</td>
<td>1.36 Sy</td>
<td>1.00Ad</td>
<td>0.43 An</td>
<td>1.00Ad</td>
<td></td>
</tr>
</tbody>
</table>

-Relative Interaction Index (R.I.I.);>1 potentiate or synergistic=Sy
-Relative Interaction Index (R.I.I.) <1 Antagonistic=An
-Relative Interaction Index (R.I.I.) =1 Additive= no observed effect= Ad

According to Mansour et al. (2001) for biochemical analysis.

Histopathological Observations:

In control group, architecture structure of testis of rats revealed multiple rounded seminiferous tubules with regular outlines. They were lined by 4-6 layers of germinal epithelium at different stages of spermatogenesis. The interstitial spaces in–between the tubules contained Leydig cells and some capillaries. The tails of mature sperms, occupy the lumina of the seminiferous tubules Figure (1).Toxic impacts of Malathion on testis tissue revealed mild to moderate toxicity reflected by spermatogenic arrest at various stages in many seminiferous tubules, many tubules appeared with reduction in size and deformity in shape while others looked detached from their basement membrane; degenerative changes in spermatogenic lineage were detected in some seminiferous tubules. Evidence of marked congestion and dilatation of venous channels at the surrounding capsule together with focal areas of capsular thickening were detected. Interstitial vascular channels with edema and moderate reduction of Leydig cells population were noticed (Figures 2a&2b). Meanwhile, in (A) group, mild to moderate signs of toxicity has been detected, in the form of spermatogenic arrest in many seminiferous tubules together with the appearance of degenerative changes within spermatogenic lineage and reduction in Sertoli cells (Figure
3. On the other hand, mild toxic impacts of Paracetamol on testis tissues were seen mainly on the vasculature where, edema in interstitial spaces with congested dilated blood vessels and prominent dilated subcapsular vessels (Figure 4). The same pathological lesions were detected in paired treatment group with M+P; besides the occurrence of focal seminiferous tubes with sever degenerative changes, deformity in shape and spermatogenic arrest as shown in Figure 5. Concerning the effect of paired treatment of (M+P), more toxic signs was observed; where the incidence and severity of the previously noticed lesions were significantly increased. Many seminiferous tubules were detached from their basement membranes, Figure 6. (A+P) group revealed moderate to severe signs of toxicity where, most of seminiferous tubules displayed spermatogenic arrest together with giant cells occasionally seen, Figures 7a&7b. The most toxic impacts were seen in case of combined treatment (M+A+P), where most of seminiferous tubules displayed various degree of spermatogenic arrest. Also, the existing spermatogenic lineage showed variable degrees of degeneration, detachment with prominent edema in interstitial spaces (Figure 8).

Plate 1: Testis tissue of albino rat treated with different environmental agents for 4 weeks using H&E; (fig1) testis tissue of control animal, showing intact seminiferous tubules (Arrow), sperms in the lumen of many seminiferous tubules, X:100. (fig 2a) testis tissue of Malathion-treated animal, demonstrating focal thickening of the capsule (Arrow), detached of some seminiferous tubules (ST), congestion of subcapsular blood vessel (Double Arrow), X: 100. (fig 2b) testis tissue of Malathion-treated animal, showing spermatogenic arrest in some of the seminiferous tubules (ST), degenerated spermatocytes, X: 400. (fig 3) testis tissue of Arsenic-treated animal showing many seminiferous tubules with irregular outlines, and spermatogenic arrest (ST), congested interstitial blood vessel (Arrow), X:40. (fig 4) testis tissue of Paracetamol-treated animal, showing desquamated cells in the lumen of some seminiferous tubules (ST) and detached from their basement membranes (Arrow), X:100. (fig 5) testis tissue of (M+A) treated animal, demonstrating degenerative changes in spermatogenic lineage (Arrow), X:400. (fig 6) testis tissue of (P+M) treated animal, showing sever degenerative changes in spermatogenic lineage (Arrow) with eosinophilic-like material deposits within the degenerated cells (Arrow head), X:400. (fig 7a) testis tissue of (P+A) treated animal, demonstrating giant cell (Arrow) in the lumen of seminiferous tubules (ST), X: 400. (fig 7b) testis tissue of (P + A) treated animal, showing severe spermatogenic arrest in many seminiferous tubules (ST) X: 100. (fig 8) testis tissue of (M+A+P) treated animal, showing seminiferous tubules with spermatogenic arrest (ST), degenerated spermatocytes (Arrow), edema in interstitial space and fibrosis (Double Arrow), X: 400.

Changes in thyroid tissues were demonstrated in plate (2); control group, showed follicles varying in size, each follicles lined with simple cuboidal epithelium (thyrocytes), having vesicular nuclei and prominent nucleoli. The follicles lumen filled with acidophilic homogenous colloid secretion having peripheral vaculations, the thyroid follicles surrounded with vascular connective tissues stroma (Figure1). In Malathion treated group, microscopical examination revealed moderate toxicity, displayed by the presence of many cystic dilated follicles with disfunctioning thyrocytes reflected by their vacuolated cytoplasm and flattened nuclei, remarkable depletion of colloid in 25% of the animal within the group, together with focal disrupted architecture and sloughed of epithelial cells in the lumen of some follicles(Figure2). Arsenic-treated group showed significant toxic impact in (80%) of the animal where many follicles with sloughed cells in their lumen, notable colloid depletion, together with focal areas of involuted follicles as shown in Figure (3). In P treated group,
mild toxic signs were observed; however, the most striking observation was the hyperplasia of the interfollicular cells together with dilated congested inter-follicular blood vessels (Figure 4). Combined treatment with Malathion and Arsenic (M+A) resulted in moderate toxicity, where some thyrocytes with vacuolated cytoplasm and flattened nuclei, this is beside few follicles with shed cells in the lumen, together with interfollicular cells hyperplasia (Figure 5). Meanwhile, the combined treatment with (A+P) produced moderate to severe toxic impact, as in (66%) of the animals, focal areas of collapsed and involuted follicles were seen, many follicles with cystic dilatation, together with prominent cytoplasmic vacuolation of most of thyrocytes lining the follicles with pyknotic nuclei, these alterations accompanied with interstitial inter-follicular aggregates and dilated blood vessels Figures (6a&6b). The administration of Malathion concomitant with P produced no more pathological alteration observed in P with A, but to lesser extent (Figure 7). The pathological observation in the animal group exposed to the combined agents (M+A+P) revealed sever toxic impact, in the form of many cystic inactive follicles, some of them with shed cells in their lumen, in addition to sever colloidal depletion, together with dilated blood vessel in the interstitial spaces as shown in Figures (8a&8b).

Plate II: Thyroid tissue of albino rat treated with different environmental agents for 4 weeks using H&E; (fig 1) thyroid tissue of control animal showing normal thyroid follicles filled with colloid (C), X: 40. (fig 2) thyroid tissue of Malathion–treated rat, showing some follicles with shaded epithelial lining (arrow), other follicles depleted from colloids (C) flattened epithelial lining (arrow head), X: 100. (fig 3) thyroid tissue of Arsenic–treated rat, showing distorted architecture, follicles with depleted colloids (F), X: 40. (fig 4) thyroid tissue of Paracetamol–treated rat, showing involutes follicles (F1), colloidal depletion in other follicles (F2), vacuolated cytoplasm of epithelial lining many follicles (arrow), X: 100. (fig 5) thyroid tissue of (M + A) treated rat, demonstrating many involutes follicles (F1), aggregates of parafollicular cells (arrow), follicles (F2) with colloid depletion, X: 100. (fig 6a) thyroid tissue of (A+P) treated rat, showing focal area of thyroid follicles with degenerated cells shed in the lumen (arrow), degenerated thyrocytes with flattened nuclei (double arrow). X: 100. (fig 6b) thyroid tissue of (A+P) treated rat, showing many thyrocytes with flattened nuclei colloid(c) with moderate decrease interfollicular cells (arrow), X: 100. (fig 7) thyroid tissue of (M+P) treated animal, showing focal distorted architecture with many follicles with shaded epithelial lining (arrow), distented follicles (F1), involuted follicles (F2), dilated interstitial blood vessel (arrow), X: 40. (fig 8a) thyroid tissue of (M+P+P) treated animal demonstrating focal areas of involuted follicles (arrow) many distented follicles (F1) X: 100. (fig 8b) thyroid tissue of (M+A+P) treated animal showing follicles with flattened thyrocytes and pyknotic nuclei, depleted colloid (C), X: 400.
Morphometrical Results:

The number of Leydig cells showed significant decrease among all treatments under investigation. Animals treated with Malathion alone as well as arsenic binary treatments showed the most affected ones in comparison to control. The height of follicular cells decreased significantly among all groups. On the other hand, follicular diameter showed a significant increase due to Paracetamol alone as well as to Malathion binary treatment sat P value < 0.05 as shown in Table 6.

Table 6: Morphometric analysis for testis and thyroid gland of adult white male rats exposed to different treatments for four weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Testis</th>
<th>Thyroid gland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of leydige cells</td>
<td>Height of follicular cells (µ)</td>
</tr>
<tr>
<td>C</td>
<td>49.48±7.24</td>
<td>99.80±1.15</td>
</tr>
<tr>
<td>M</td>
<td>12.31±1.10</td>
<td>64.70±0.94</td>
</tr>
<tr>
<td>A</td>
<td>18.03±2.20</td>
<td>63.26±1.40</td>
</tr>
<tr>
<td>P</td>
<td>16.71±2.60</td>
<td>64.88±1.48</td>
</tr>
<tr>
<td>M+A</td>
<td>21.53±1.80</td>
<td>66.69±1.9</td>
</tr>
<tr>
<td>M+P</td>
<td>21.02±0.60</td>
<td>57.20±0.70</td>
</tr>
<tr>
<td>A+P</td>
<td>17.40±3.00</td>
<td>51.80±0.81</td>
</tr>
<tr>
<td>M+A+P</td>
<td>18.81±2.20</td>
<td>60.50±0.73</td>
</tr>
</tbody>
</table>

- Results were expressed as mean ± SE for each 7 rats.
- * Significance difference versus control at P < 0.05.
- a, b, c Significance difference of the mixture versus M, A, P, respectively at P < 0.05.

Discussion:

A growing concern was indicated of public health about long-term use of Paracetamol, as efficient pain release belonging to nonsteroidal anti-inflammatory drugs and low-level exposure to different environmental factors. In the current study, we evaluated the hormonal disruption, physiological and oxidative stress changes to concurrent subchronic exposure to Malathion, Arsenic and Paracetamol individually or in combination. Our findings revealed that co treatment with (M+ A+ P) induced significant reduction in body weight which is considered as a simple and sensitive index of toxic effects (Lu, 1996). The calculations of the joint action analysis that indicated the potentiation of the combined agents against growth rate than that observed in individual treatment groups confirmed our finding and assessed that mixture toxicants may interact differentially with various biological aspects of the subjected organism.

Regarding thyroid gland biomarkers T3& T4, the current results revealed that combined treatment with Paracetamol with other agents’ induced significant decrease in T3 level as compared to control and individually treated groups. These results concomitant with significant increase in T4 level in the same groups, supported antagonistic effect of these agents against T3& T4 as confirmed by joint action analysis, as well as degeneration in thyroid follicles demonstrated by histopathological examination.

Previous reports had revealed that pesticide exposure in agriculture workers induced disturbances of thyroid hormone levels (Zaidi et al., 2000; Garry et al., 2003; Toft et al., 2006). Also, Arsenic injured thyroid follicles and altered the synthesis as well as the secretion of thyroglobulin and oxidized iodides from thyroid gland (Wu et al., 2008). It is known that prostaglandins inhibitors, like Paracetamol, inhibit cAMP activity as well as inhibit the stimulatory effect of TSH on thyroid gland and caused decrease in biosynthesis of T3 and T4 (Boeynaems et al., 2002). Decreased hepatic metabolism of thyroid hormones may be one mechanism responsible for the increased level of T4 in the present study. Thus, inhibition of the enzymes involved in thyroid hormone metabolism such as 5′-deiodinase type II (D2) may lead to decreased synthesis of T3 from T4 and consequently an accumulation of T4 in serum. This mechanism could be supported by the present reduction in T3 levels and runs in parallel to that recorded by Lacasana et al. 2010. The present work showed responses to the impairment of thyroid function by contaminant exposure which may be critical to young and growing individuals, with severe consequences including growth retardation (Schantz and Widholm 2001; Smits et al., 2002; Vos et al., 2000).

Also, the current study revealed different levels of significant decrease in Testosterone level associated with reduction in Leydig cells numbers as well as spermatogenic arrest, edema in interstitial spaces in testis, which was coupled with increased relative weight of testis after individual treatments. The concurrent exposure of (M+A) and (A+P) were accounted to antagonistic effect while the other combined treatments showed synergistic effect. Previous studies revealed that Malathion can induce human birth defects (Lindouout, 1987) and has been known as endocrine disruptor (Ishihara et al., 2003; Gwinn et al., 2005), as it induced degenerative effects on testes when given orally to mice leading to their infertility (Kumar and Nath, 1997). Our findings were in accordance with (Balasubramaniam et al., 1987; Zhang et al., 2007; Choudhary et al., 2008; Uzun, 2009), who stated that exposure to Malathion and other synthetic insecticide dramatically reduces testosterone level. In contrary, other organophosphate pesticides increase testosterone level in rats (Moustafa et al., 2008).
Decreased testosterone level might be attributed to the inhibitory effect of Malathion on the secretion of pituitary gonadotrophins and on turns on the testosterone biosynthesis (Uzun, 2009). This effect may lead not only to decreased Leydig cells numbers but also to the pathological changes in the leydig cells encountered in the present investigation. Arsenic is a potent endocrine disruptor, because it alters steroid and thyroid hormone receptor-mediated gene regulation at low and environmentally-relevant levels in cell culture and whole-animal models (Kaltreider et al., 2001; Bodwell et al., 2004, 2006; Davey et al., 2007, 2008). Additionally, Arsenic has a suppressive influence on spermatogenesis and gonadotrophin and testosterone release in rats (Sarkar et al., 2003). Also, rodent model experiments have indicated that Arsenic impairs male reproductive functions by inducing oxidative stress (Chang et al., 2007). It has been also demonstrated that a number of putative endocrine disrupting compounds, which are known to be anti-androgenic and potentially exert anti-masculinization effects in humans (Swan et al., 2005) are potent inhibitors of prostaglandins (PG) synthesis in both rodent and human cells (Kristensen et al., 2011; 2012). This could suggest that these endocrine disrupting compounds can damage male reproductive function by inhibiting PG synthesis. This hypothesis is supported by the previous studies that mild analgesics including Paracetamol which relieve pain and reduce inflammation by reducing PG synthesis may reduce testosterone production (Gupta and Goldman, 1986; Gupta, 1989).

Oxidative stress is a major mechanism in the pathophysiology of several toxins and diseases. Meanwhile, it is also a process related to xenobiotic exposure and different levels of environmental contamination (Banerjee et al., 1999). The present study revealed marked disruption in the oxidative stress markers as evidenced by a significant decrease in total antioxidant capacity associated with significant increase in malondialdehyde MDA level (the biomarker of lipid peroxidation), among all groups in comparison to the control group. The explanation for these findings was previously attributed to the long-lasting exposure to M, A and P individually or in combinations that could lead to generation of reactive oxygen species which simply consume and exhaust antioxidant agents present in the body (Gupta et al., 1992). The results of joint action analysis, co-exposure revealed the marked incidence of changes against MDA, TAC and total protein content after individual and combined treatments. These results run partially in parallel with the previous study by Nabi et al. (2005) who recorded elevated total protein level in Bengal population with chronic Arsenic exposure. In some inflammatory conditions the release of tumor necrosis factor induces increase synthesis of proteins of the acute phase response (Srivastava et al., 2009). Recently, animal studies reported that exposure to either Arsenic; pesticide or Paracetamol induced systemic inflammation and enhanced tumor necrosis factor release (Das et al., 2012; Mecdad et al., 2011; Ghosh et al., 2010).

Finally and from the results obtained, it may be reported that the repeated co-exposure to Malathion, arsenic and Paracetamol (M+A+P) for 28 days in male rats were more hazardous to body weight, as classical toxicological end point and testosterone activity (synergistic), but effects on T3, T4 and TAC appeared to be antagonistic. On the other hand, MDA and total protein remained almost parallel to those produced by the individual toxicant (additive). Therefore, the misuse of Paracetamol in addition to exposure of xenobiotics should be of special concern in worldwide, and it is paramount importance to further assess of their mechanism of action to understand and predict their overall health impact.

References


