Bioremediation of Contaminated Diets with Industrial Wastewater – Phenol Compounds Using Marine and Earth Fungi: DNA Fragmentation, Cytogenetic and Sperm Studies in Mice

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Abstract: In the present work, DNA fragmentation, cytogenetic and sperm studies were used to evaluate the bioremediation of contaminated diets with wastewater – phenol compounds derived from a sugarcane bagasse cellulose wood-mill (SBCW-mill) by marine (Aspergillus oryzae) and earth (Trichoderma reesei) fungi. The discharged wastewater samples were used for preparing the biological diets. Five experimental groups of mice were used. The first group was fed a normal diet (N diet) and used as control. The second group was fed the same normal diet mixed with manufactory discharge (ND diet). The third, fourth and fifth groups were fed ND diet treated with marine (A. oryzae) fungus (NDA diet), earth (T. reesei) fungus (NDT diet) and mixture of marine and earth fungi (NDAT diet), respectively. The animals of the five groups were fed the corresponding mentioned diets for three weeks. After that, mice were inspected for genetic and sperm studies. The present results showed that the feeding on contaminated diets with wastewater – phenol compounds (ND diet) induced significant increases of frequencies of each of DNA fragmentation and micronucleated erythrocytes as compared to feeding on normal diet. Also, ND diet caused significant elevation of structural and numerical chromosome aberrations. Moreover, a significant increase in the number of morphologically abnormal sperms and a significant decrease in sperm count were occurred in animals fed ND diet as compared to those found in control group. However, the use of treatment with A. oryzae (NDA diet) or T. reesei (NDT diet) or mixture of A. oryzae plus T. reesei diminished most of the deleterious effects of ND diet alone and significant enhanced the genetic and sperm measurements. The ability to ameliorate such measurements was more effective in treatment with T. reesei than treatment with A. oryzae. Furthermore, the treatment with mixture of A. oryzae plus T. reesei was more pronounced for improvement of genetic and sperm parameters than A. oryzae or T. reesei treatment alone, where this NDAT group had the lowest percentages of DNA fragmentation, micronuclei, chromosome aberrations and sperm abnormalities as well as it had the highest proportion of sperm count. In conclusion, the present study confirmed that using mixture of A. oryzae plus T. reesei is much better than using A. oryzae or T. reesei alone for bioremediation of contaminated diets with wastewater-phenol compound. This strategy is necessary for clean up the Egyptian environment from industrial- wastewater pollution.

Key words: Aspergillus oryzae • Trichoderma reesei • Phenolic industrial wastes • DNA fragmentation • Micronucleus • Chromosome aberrations • Sperm abnormalities

INTRODUCTION

Phenols and their derivatives commonly present in the environment and human surroundings [1, 2]. They occur in the wastewater of many industrial process including oil production, coal conversion, paper production and are also widely used in consumer products and pharmaceuticals [3]. The presence of phenols in the ecosystems is also related with production and degradation of numerous pesticides and the generation of municipal sewage, in particular phenoxyherbicides like 2,4 dichlorophenoxyacetic acid.
(2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MCPA) and also phenolic biocides like pentachlorophenol (PCP) [4-6]. Some phenols are also formed during natural processes like the formation of phenol and p-cresol during decomposition of organic matter or synthesis of chlorinated phenols by fungi and plants [7]. These compounds may be substituted with chlorine atoms, nitratred, methylated or alkylated that are harmful ecotoxins [2]. Toxic action of these compounds stems from unspecified toxicity related to hydrophobicity and also to the generation of organic radicals and reactive oxygen species (ROS) [8, 9]. Moreover, phenols reveal peroxidative capacity, they are hematotoxic and hepatotoxic, provoke mutagenesis and carcinogenesis toward humans and other living organisms [2]. For instance, phenols after penetration of the cell, undergo active transformation, mainly at the participation of oxidases within cytochrome P450. Sometimes transformation processes lead to increase of toxicity of individual compounds by the formation of electrophilic metabolites that may bind and damage DNA or enzymes. The noxious influence of phenols and their derivatives concern acute toxicity, histopathological changes, mutagenicity and carcinogenicity [2].

In Egypt, characterizations of cellulose from sugarcane bagasse have become an important source of wood industry. The wastewater of this industrial unit contains phenol or phenolic compounds, as phenol is involved in some stages of the manufacturing process. The disposal of phenol-containing wastewater without proper treatment causes negative effects on the environment, since phenols or phenolic compounds are defined as one of the priority pollutant and is not easily degradable [10].

Efforts are now being made to remove organic contaminants, as much as possible without external resources of energy. In this respect, the use of biological methods is considerable [10,11]. Fungal strains are playing a very important role in the degradation of organic compounds. For example, most of decolorization studies have been demonstrated using the white rot fungi, which were able to degrade a broad spectrum of dyes [11]. Also, fifteen fungal strains were isolated from arsenic contaminated agricultural soils from the state of West Bengal, India and it was reported that the most effective removal of arsenic was observed in the fungal strains, Trichoderma Sp. and it was recommended that these fungal strains can be effectively used for the bioremediation of arsenic-contaminated agricultural soils [12]. In addition, A. oryzae is a safe well defined Aspergillum, because it is capable of converting complex organic molecules to simple one and increasing the bioavailability of phosphate as energy source into the surrounding environment [13, 14]. Moreover, Aspergillus oryzae (marine fungus) and Trichoderma reesei (earth fungus) could be considered as important strains in biological tests. These fungi have not been pathogenic or toxic agents for plants or animals [15-18]. A. oryzae has been an essential part of the oriental food production such as soy sauce, sake kji and miso [13, 14, 19]. Also it is used to produce livestock probiotic feed supplements [13, 14]. On the other hand, T. reesei enzyme preparations have a history of safe use in many industries including starch and animal feed processing, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper and textiles [14, 15].

So, the present study was designed to evaluate the bioremediation of contaminated diets with wastewater – phenol compounds derived from sugarcane bagass cellulose wood-mill (SBCW-mill) by marine (Aspergillus oryzae) and earth (Trichoderma reesei) fungi. DNA fragmentation, cytogenetic and sperm studies were investigated in mice as parameters for this purpose.

**MATERIALS AND METHODS**

**Wastewater Samples Collection:** Samples of wastewater were collected from a sugarcane bagass cellulose wood-mill (SBCW-mill) this manufactory is located at one of the Governorates of Upper Egypt. Samples were collected in plastic vessels, transported to the laboratory immediately after sampling and stored at 4°C. The phenol compounds were the main pollutants in the wastewater samples. The concentrations of phenol compounds (Table 1) were determined by the Water Pollution Department, National Research Center, Egypt, according to the standard methods [20].

**Microorganisms:** Aspergilus oryzae (Marine fungus) and Trichoderma reesei F=418 (Earth fungus) were obtained from the Microbial Chemistry Department, N.R.C., Egypt. - Diets: 12.5 kg of the basal diet were obtained from the Animal House, N.R.C., Egypt. This diet meets the mice nutrient requirements according to the National Research Council (NRC) [21].

**Normal Diet:** A quantity (2.5 kg) of basal diet was used as normal diet (N diet) or control.
Table 1: Concentration of the phenol compounds in the wastewater samples derived from SBCW-mill

<table>
<thead>
<tr>
<th>Phenol compounds</th>
<th>Concentration (ug / L)</th>
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<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>Nd</td>
</tr>
<tr>
<td>2- Nitrophenol</td>
<td>Nd</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>460.82</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>Nd</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>129.74</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>624.87</td>
</tr>
<tr>
<td>4- Nitrophenol</td>
<td>Nd</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>564.94</td>
</tr>
<tr>
<td>Phenol</td>
<td>Nd</td>
</tr>
</tbody>
</table>

Total phenol compounds 1779.87 µg / L = 1.78mg/L
Nd = not detected

Discharged Wastewater Diet: The remaining quantity (10kg) of basal diet was mixed with 5 liters discharged wastewater (ND) from SBCW-mill (50%W/W diet). Thus, the concentration of phenol compounds was considered to be about 890 µg/kg diet or (8.9 µg/10 gm diet). The daily intake from the diets was about 10 gm / mouse. So, the daily intake from the phenolic compounds was considered to be about 8.9 µg / about 30 gm of body weight. Such concentration of phenolic compounds is very toxic and affects animal organs [2, 8 and 22]

Biological Diets: Discharged wastewater diet (ND) was divided into four equal section (2.5 kg each). The first section was used as positive control. The second, third and fourth sections were treated with *Asperigillus oryzae* (NDA diet), *Trichoderma reesei* F-418 (NDT diet) and a mixture of *Asperigillus oryzae* plus *Trichoderma reesei* F-481(NDAT diet), respectively. The treatment with fungi was according to Farag et al. [17] and the preparation the biological diets can be shown as follows: *Asperigillus oryzae* or *Trichoderma reesei* F = 418 were cultured separately in PDA (potato dextrose agar) medium for 3 days. After that they were separately crushed in 15ml of sterilized water with 0.01 Tween 80 and shaked for 10 minutes. Fungus spores from the two above fungi were used separately or in combination as an inoculum at 10% (v/w) to inoculate separately cooled sterilized (Autoclaved at 121°C for 30 minutes) three conical flasks. The capacity of each flask was 250ml. Each flask contained 10g of mice diet (Mixed with discharge of manufactory) which was moistened with water, to be about 65% humidity. The inoculated flasks were incubated at room temperature 28°C ± 2 for 5 days. The obtained growth was used to treat mice diet 10% (w/w) and then incubated for 5 days. At the end of incubation period, the treated mice diets were air dried, then used in the formula diet.

Experimental Animals: Fifty male Swiss Albino mice (Mus musculus) weighing 25-30 grams were obtained from the Animal House at the National Research Center, Giza, Egypt. They were kept in an ambient temperature of 25 ± 3.2°C on light/dark cycle of 12/12 hours and supplied with fresh water *ad- libitum*.

Experimental Design: Mice were randomly divided into five groups (10 mice for each). The first group was fed a normal diet and used as a negative control (N diet). The second group was fed normal diet mixed with manufactory effluent discharge (ND diet) and employed as a positive control. The third, fourth and fifth groups were fed normal diet mixed with manufactory effluent discharge and treated with marine fungus (NDA diet), earth fungus (NAT diet) and mixture of marine and earth fungi, (NDAT diet), respectively. The animals of all groups were fed the corresponding, previously mentioned, diet for 3 weeks (about 10 gm diet daily/animal). After that mice were tested for DNA fragmentation, cytogenetic analysis (micronucleus test and chromosome aberrations) and sperm abnormalities.

DNA Fragmentation: Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5ml lysis buffer containing 10mM tris-HCl (pH, 8) 1mM EDTA, 0.2% triton X-100 centrifuged at 10000 r.p.m. (Eppendorf) for 20 minutes at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and supernatant (S), 1.5 ml of 10% trichloroacetic acid (TCA) was added and incubated at 4°C for 10 minutes. The samples were centrifuged for 20 minutes at 10000 r.p.m. (Eppendorf) at 4°C and the pellets were suspended in 750 ul of 5% TCA, followed by incubation at 100°C for 20 minutes. Subsequently to each sample 2 ml of DPA solution [200 mg DPA in 10 ml glacial acetic acid, 150 ul of sulfuric acid and 60 ul acetaldehyde] was added and incubated at room temperature for 24 hour [23]. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

\[
\text{DNA fragmentation} = \frac{\text{OD of fragmented DNA (S)}}{\text{OD of fragmented DNA (S)} + \text{OD of intact DNA (P)}} \times 100
\]

Cytogenetic Analysis

Micronucleus Test: Bone marrow slides were prepared according to the method described by Krishna and Hayashi [24]. The bone marrow was washed with 1 ml of fetal calf serum and then smeared on clean slides. The slides were left to air dry and then fixed in methanol for 5 minutes, followed by staining in May-Grunwald-
Giemsa for 5 minutes then washed in distilled water and mounted. For each animal, 2000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei.

Chromosome Preparations: For chromosome analysis both treated and control animals were sacrificed by cervical dislocation at the end of experiment. One hour and half or two hours before sacrifice, mice were injected with 4 mg colchicine / kg. b.w. Femurs were removed and the bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method of Preston et al. [25]. Fifty metaphase spreads per animal were analyzed, for scoring the different types of chromosome aberrations.

Sperm Analysis: For sperm-shape analysis, the epididymus excised and minced in about 8 ml of physiological saline, dispersed and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with Eosin Y (aqueous), according to the methods of Wyrobek and Bruce [26] and Farag et al. [27]. At least 3000 sperms per group were assessed for morphological abnormalities. The sperm abnormalities were evaluated according to standard method of Narayana [28]. Epididymal sperm count was also determined by hemocytometer as described by Pant and Srivastava [29].

Statistical Analysis: Statistical analysis was performed with SPSS software. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for comparison between different treatments. Results were reported as mean ± S.E. and differences were considered as significant when P < 0.05.

RESULTS

DNA Fragmentation: The present results (Table 2) showed that the rates of DNA fragmentation significantly (P<0.001) increased in animals fed ND diet (positive control) than those found in animal fed N diet (negative control). Whereas, the rates of DNA fragmentation significantly (P<0.05, P<0.01 or P<0.001) decreased in mice fed NDA, NDT or NDAT diets in comparison with those found in ND group. These decreases were more pronounced in the NDT group than in NDA group. Moreover, the treatment with mixture of Aspergillus oryzae plus Trichoderma reesei (NDAT diet) was more effective in decreasing (P<0.01 or P<0.05) the rates of DNA fragmentation than treatment with Aspergillus oryzae or Trichoderma reesei alone.

Micronucleus Assay: As shown in Table 3, the frequencies of micronuclei were significantly (P<0.0001) increased in mice fed ND diet as compared to those observed in animals fed N diet. Whereas, the frequencies of micronuclei significantly decreased (P<0.05, P<0.01 or P<0.001) in mice fed NDA, NDT or NDAT diets in comparison with those observed in positive control (ND diet). The reduction of micronuclei was more obvious in NDT group than those found in NDA group. On the other hand, the treatment with mixture A. oryzae plus T. reesei (NDAT diet) caused a significant (P<0.01 or P<0.05) reduction in the frequencies of micronuclei than neither NDA nor NDT alone. However the aberrations which observed in NDAT group was still significantly (P<0.05) higher than those found in the negative control (N group).

Table 2: DNA fragmentation in male mice fed different diet.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate of DNA fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N diet</td>
<td>13.99±0.72d</td>
</tr>
<tr>
<td>ND diet</td>
<td>59.46±2.81e</td>
</tr>
<tr>
<td>NDA diet</td>
<td>49.32±1.63b</td>
</tr>
<tr>
<td>NDT diet</td>
<td>42.41±1.51c</td>
</tr>
<tr>
<td>NDAT diet</td>
<td>18.92±0.61d</td>
</tr>
</tbody>
</table>

N=Normal diet (negative control).
ND=Discharge diet: normal diet mixed with wastewater of SBCW-mill (positive control).
NDA=ND diet treated with A. oryzae.
NDT= ND diet treated with T. reesei.
NDAT= ND diet treated with both A. oryzae and T. reesei.
a,b,c,d means with different letters are significantly different (P<0.05).
All data are expressed as means ± SE.

Table 3: The frequencies of micronucleated polychromatic erythrocytes (MNPCE) in male mice fed different diets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of examined cells</th>
<th>Mean values of MNPCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N diet</td>
<td>20000</td>
<td>2.00±0.41e</td>
</tr>
<tr>
<td>ND diet</td>
<td>20000</td>
<td>20.50±0.64a</td>
</tr>
<tr>
<td>NDA diet</td>
<td>20000</td>
<td>16.50±0.64b</td>
</tr>
<tr>
<td>NDT diet</td>
<td>20000</td>
<td>13.50±0.64c</td>
</tr>
<tr>
<td>NDAT diet</td>
<td>20000</td>
<td>6.75±0.48d</td>
</tr>
</tbody>
</table>

N=Normal diet (negative control).
ND=Discharge diet: normal diet mixed with wastewater of SBCW-mill (positive control).
NDA=ND diet treated with A. oryzae.
NDT= ND diet treated with T. reesei.
NDAT= ND diet treated with both A. oryzae and T. reesei.
a,b,c,d,e means with different letters are significantly different (P<0.05).
All data are expressed as means ± SE.
Chromosome Examination: Examination of the mouse chromosomes showed structural and numerical aberrations (Table 4) Structural chromosome aberrations included chromatid gaps and breaks, deletions, fragments, centromeric attenuations (C.A.), and endomitosis (End.). Numerical chromosome aberrations were aneuploidy and polyploidy. The results showed that, ND group had higher frequencies of structural and numerical aberrations than N group (control). Statistical analysis showed significant difference between ND and N groups for the frequencies of structural (except fragments) and numerical chromosome aberrations. The mice fed NDA, NDT or NDAT diets had decreases of structural (Except fragments of NDT and NDAT groups and deletions of NDA group) and numerical aberrations as compared to mice fed ND diet alone. These decreases were significant for the frequencies of structural (Except chromatid breaks and fragments of NDAT group, chromatid breaks of NDT and NDAT groups) and numerical (Except aneuploidy of NDA group) chromosome aberrations. Moreover, NDT group had significant (P<0.05) decreases of total structural chromosome aberrations and aneuploidy as compared to NDA group, while NDA group had the lowest (P<0.05) frequencies of polyploidy. Also, NDAT group had significant (P<0.01 or P<0.05) reduction for the frequencies of total structural and total numerical chromosome aberrations as compared to NDT or NDA groups.

Sperm-Shape Analysis: Sperm examination (Table 5) showed that the head and tail sperm abnormalities were more frequent in ND group than those of N group (control). Statistical analysis indicated that there were significant differences for the frequencies of head and tail abnormalities between ND and N groups. Exception to this the frequency of big heads non-significant increase in ND group in comparison with those found in N group. The treatment with NDA, NDT or NDAT diets led to decrease of the frequencies of head and tail sperm abnormalities as compared with ND alone. These decreases were significant (P<0.05 or p<0.01) for all frequencies of head and tail abnormalities. Exception to this, the frequencies of big and banana heads in NDA, NDT and NDAT groups and the frequency of coiled tail abnormality in NDA group non-significantly decreased than those found in ND group. On the other hand, NDT treatment was more effective for decreasing the frequencies of sperm abnormalities as compared to NDA treatment, there were significant differences (P<0.05) for frequencies of total head (especially amorphous) and total sperm abnormalities between the two treatments.

Furthermore, the use of NDAT diet was more pronounced for reduction of the sperm abnormalities than NDA or NDT diets and there were similar significant differences (P<0.05) for the frequencies of sperm abnormalities (except big and banana head abnormalities and coiled tail abnormality) between NDA and NDAT groups or between NDAT and NDT groups.
Sperm Count: From the present results (Table 5), it was found that sperm counts significantly decreased (P<0.001) in mice fed ND diet than those found in the control. In contrast, the treatments with NDA, NDT or NDAT enhanced the results and gave significant (P<0.01 or P<0.05) increases of sperm counts as compared to ND treatment alone. The results showed that there were no significant differences for sperm count between NDT or NDA groups. On the other hand, the sperm counts were significantly (P<0.05) elevated in NDAT group in comparison with those found in either NDA or NDT groups.

DISCUSSION

The present results showed that the feeding on contaminated diets with industrial wastewater-phenol compounds (ND diet) induced significant increases of each of DNA fragmentation and micronucleated erythrocytes as compared to feeding on normal diet (control). Also, ND diet caused higher frequencies of structural and numerical chromosome aberrations. These findings were supported by report of Paintner and Howard [30] who revealed the mutagen activity of phenols by observation the inhibition in synthesis and replication of DNA in Hela cells. Yiqiang et al. [3] found that the treatment with phenol led to significant increases of micronucleus (MN) frequencies (P<0.05 or P<0.01) in human lymphocytes and mouse spermatids in comparison with control. Moreover, in previous study, Zhang et al. [31] reported that the exposure to phenols stopped reparation of DNA in diploid human fibroblasts and induced damage in human lymphocyte chromosomes by increasing deletion ratio in 7. chromosome, which may lead to leukemia development.

The mutagenic effects of phenols might be related to their toxicity that could be expressed in the cells by forming reactive oxygen species (ROS) and lipid peroxidation (LPO) during the metabolic processing of phenol in liver [2]. ROS include a hydroxyl radical that can interact with genomic DNA leading to anomalies in DNA and consequently cause the impairment of cell functionally, cytotoxicity and genotoxicity [2, 32, 33], that have been confirmed in our findings by the presence of more frequencies of DNA fragmentation, micronuclei and chromosome aberrations in phenols group than those of control group.

On the other hand, micronucleus assaying as of chromosome aberrations is a cytogenetic form that measures chromosomal damage, thus it is only effective when both DNA strands are broken [34-36]. Therefore, the significant increases of micronuclei which observed in the present study proved also the mutagenic effect of phenol compounds that consequently led to inducing of high frequencies of chromosome aberrations in phenols group.

In the present study a significant increase in the number of morphologically abnormal sperms and a significant decrease in sperm counts occurred in animals fed ND diet. The consequently high incidence of DNA fragmentation and chromosome aberrations as a result of potential generation of ROS during metabolic processing of phenols in liver may be indicative of a general susceptibility of animals in the present study for inducing DNA fragmentation and consequently chromosome anomalies in gonadal cells causing sperm shape abnormalities and reduction of sperm counts [37, 38].

Sperm abnormalities in phenols group might be also due to attack of generated ROS to polyunsaturated fatty acid residues of phospholipids of cell membrane occurring lipid peroxidases (LPO). Since the sperms have a high content of polyunsaturated fatty acids in the plasma membrane they are highly sensitive to oxidative stress. Increased LPO and altered membrane can affect the sperm DNA leading sperm abnormalities [39, 40].

Evidence that sperm shape abnormalities induced by selected mutagens and carcinogens, have been reported by Wyrobek et al. [41] and Sinha and Prasad [42]. DNA fragmentation of human genomic of gonadal cells was due to an excessive production of ROS by oxidative stress and lead to damage in sperm morphology [38, 43].

On the other hand, our results might be in coincidence with those of Aoyama et al. [44] and Jung et al. [45] who stated that phenols are capable of disturbing sexual hormones function, which finally may lead to sterility of animals and humans. The examples are alkylphenols, bisphenol A, 2, 4-dichlorophenol and pentachlorophenol. Also, Niwa-Kuruto et al. [46] reported in another experiment that bisphenol A caused protein expressions in Tm4 cells in mice, which play a key role in spermatogenesis, leading to decrease of viability of cells from 10 to 70% and inducing infertility in mice.

The Bioremediation with Fungi: The present results showed that the treatment with A. oryzae (NDA diet) or T. reesei (NDT diet) or a mixture of them (NDAT diet) was able to significantly decrease the rate of DNA fragmentation, the frequencies of micronuclei, proportion of chromosome aberrations and percentage of sperm abnormalities as well as significantly increase of the sperm...
counts as compared to phenols treatment (ND diet) alone. The ability to ameliorate the parameters of genetic materials and sperm shape as well as sperm count was more effective in the treatment with T. reesei than treatment with A. oryzae. Moreover, the treatment with mixture of T. reesei plus A. oryzae was more pronounced for improving of all above parameters than T. reesei or A. oryzae treatment alone. These findings were supported by report of Eshak et al. [47] who found by using comet assay that DNA damage which observed in mice fed diet contaminated with phenols (ND group) significantly decreased in NDA, NDT and NDAT groups and they observed that NDT treatment was better than NDA treatment for decreasing of such aberration and NDAT group had the lowest percentage of DNA damage. Also, in previous study Farag et al. [17] found that mice fed basal diet treated with T. reesei had low frequencies of chromosome aberrations in somatic and germ cells and high percentage of mitotic index in comparison with mice fed basal diet (control) alone. Furthermore, Shoman and Fadel [18] found that the biologically treated basal diets with T. reesei fungus did not have any genotoxic activity on somatic and germ cells in rats and they reported that this fungus can be added in the ratio of farm animals without any hazardous effects.

The improvement of genetic materials and sperm shape as well as sperm count in this study demonstrates that fungal strains (especially the mixture of A. oryzae plus T. reesei) can be effectively used for the bioremediation of contaminated diets with industrial wastewater-phenol compounds. This bioremediation might be due to degradation of toxic phenol compounds by fungi. Similarly, most decolorization studies have been demonstrated using a number of fungal species such as Aspergillus fumigatus [48], Cerrena unicolor [49] and Aspergillus niger [50]. Moreover, Aspergillus oryzae is a safe well defined aspergillum, it has the capability of converting complex organic molecules to simple ones increasing the bioavailability of phosphate as energy source to the surrounding environment [13, 14]. Also, it was demonstrated that some strains of fungi can transform lead in polluted environment into its most stable mineral form, leading to keep hazardous of such mineral under control [51].

In conclusion, mixture of A. oryzae plus T. reesei is much better than using A. oryzae or T. reesei alone for the bioremediation of contaminated diets with wastewater-phenol compounds. This strategy is necessary for clean up the Egyptian environment from industrial-wastewater pollution.

REFERENCES


