Combined toxicity of endosulfan and ochratoxin-A in rats: histopathological changes

Shashi Nandar Kumar1, Banajit Bastia1, Avinash Gopal Telang2, Karam Pal Singh2, Rambir Singh3 and Arun Kumar Jain1*

Abstract
The aim of this study was to examine and compare the toxic effects of endosulfan and ochratoxin-A (OTA), individually and in combination, in adult male rats with respect to histopathological changes over 30 days of treatment. Adult male Wistar rats were randomly allotted to four groups (individual treatments, combination and control) of 10 rats each and fed OTA @ 4 ppm in feed (Group-I), endosulfan @ 5 mg/kg BW in corn oil by oral gavage (Group-II), and endosulfan with OTA in combination (Group-III) daily for 30 days. Group IV was kept as control and fed toxin free feed. After 30 days of treatment, tissues were collected in 10% buffered formalin for histopathological studies. The hematoxylin and eosin stained paraffin sections showed varying degree of degenerative and necrotic changes in kidneys and liver which were more severe in OTA treated rats in comparison to endosulfan fed group. However, the changes observed in group III rats, which received both OTA and endosulfan were even more pronounced than those observed in rats fed OTA or endosulfan alone. The present findings suggest that OTA and endosulfan had additive effects which may play an important role in pathogenesis.

Keywords: Ochratoxin-A, endosulfan, toxicity, histopathology, liver, kidney

Introduction
Mycotoxins can be found in nearly all agricultural commodities, such as cereals (maize, wheat, oats and barley) and cereal by-products. Moulds or fungi and their associated mycotoxins have been shown to be present on all crops in varying amounts, depending on the climatic conditions. Correct storage of feeds and feedstuffs is critical in preventing mould growth. While improper storage of feed stuff may result in excessive mycotoxin contamination, current agricultural practices with emphasis on widespread use of pesticides may cause pesticide contamination of agricultural items which become toxic for consumption. These contaminations can cause serious problems, both for human health as well as for the economic value of crops.

Endosulfan is a persistent, toxic, broad-spectrum organochlorine insecticide and acaricide used on food and non-food crops. It has been enlisted as one of the persistent organic pollutants (POPs) that is largely used in agriculture, viticulture and horticulture [1]. Endosulfan contamination in blood, fat, milk, vegetables, cashew leaves, soil and water has been reported from Padre village of Kasargod district of Kerala, India [2,3]. It causes toxic effect on almost all the organs of both humans and animals including the liver, lung, central nervous system, genital system, pancreas etc [4]. Endosulfan acts as an endocrine disruptor, causing reproductive and developmental damage; and has also been reported to cause immune suppression and cancer in experimental animals [1,5-10].

Ochratoxin-A (OTA) is one of most toxic mycotoxins produced by numerous varieties of Aspergillus and Penicillium [11,12]. Worldwide contamination of OTA has been reported in a wide range of foods, including cereals and peanuts [13], poultry feeds [14], feedstuffs [15], green coffee beans [16], cocoa beans [17], wine grapes (18), dried vine fruits [19] and beer [20]. It is also reported toxic manifestations of OTA include nephrotoxicity, teratogenicity, immunotoxicity, carcinogenicity, and mutagenicity [21]. OTA is suspected to be responsible for human Balkan Endemic Nephropathy (BEN) [21,22]. Thus, kidney is considered to be one of the main target organs of OTA [23]. Due to its ubiquitous occurrence in a range of foodstuffs, the complete
avoidance of OTA exposure is virtually impossible [23-25].
Further, in real life, organisms are concurrently exposed to
several i.e., more than one pollutants or toxins. Therefore, the
present study was aimed to examine the pathological changes
in liver and kidney of adult male rats in case of combined
exposure to OTA and Endosulfan.

Materials and methods
Production and analysis of OTA
Aspergillus ochraceus NRRL-3174 was initially procured from
National Centre for Agriculture Utilization Research, Peoria,
Illinois, USA. It was grown on sterilized maize as per the method
described by Trenk and coworkers [26]. The extraction and
clean up of the toxin sample were done by AOAC method
[27] and quantification of OTA was done with the help of TLC
scanner (CAMAG, Switzerland).

Preparation of toxicated feed
Cultured maize powder containing known amount of OTA
was added to basal ration (which were tested negative for
presence of contaminating mycotoxins) in such proportion
that the final concentration of OTA was adjusted to 4 mg kg⁻¹
feed. Aliquots were taken from the mixed diet and the toxin
was further quantified to ensure proper mixing of the toxin.

Dosing of endosulfan
Endosulfan (>99.98% pure crystalline Technical grade Shriram
Chemicals Ltd., India) was dissolved in corn oil (vehicle) at a con-
centration of 5 mg kg⁻¹ body weight and oral gavage was given
to male rats, daily for 30 days. Fresh solution of endosulfan was
prepared on each day of treatment. Control animals received an
equal volume of corn oil similar to those treated with endosulfan.

Experimental plan
Forty sexually mature male Wistar rats (Age: 7 to 8 weeks;
weight:180±20 g), procured from the Laboratory Animal
Resource (LAR) section of the Indian Veterinary Research
Institute were maintained on standard feed and water avail-
able ad libitum. After 1 week of acclimatization period, the
animals were randomly distributed in to 4 groups of ten rats
each and treated for 30 days as follows: Group-I animals were
given with a diet containing OTA at the level of 4 mg kg⁻¹
feed; Group-II animals received endosulfan at a concentration
of 5 mg kg⁻¹ body weight by oral gavage; Group-III animals
received both OTA (4 mg kg⁻¹ feed) and endosulfan (5 mg
kg⁻¹ body weight) and Group-IV animals were fed a standard
mycotoxins-free basal diet. All the experimental procedure
and sacrifice of rats were carried out as per the approved
guidelines of Institutional Animal Ethics Committee (IAEC)
and Committee for the Purpose of Control and Supervision
of Experiments on Animals (CPCSEA).

Pathological changes
The liver and kidney of all the groups were examined for
gross morphological and pathological changes. Wet organ
weights were recorded before collecting tissue samples for
processing. Representative tissue samples were collected
in 10% buffered formalin for histopathological studies. The
tissues were properly processed and embedded in paraffin
wax. The sections were stained with hematoxylin and eosin
stain [28] and were then examined under light microscope.

Statistical analysis
Data generated during the study were suitably analyzed using
student’s t-test to detect differences between means among
groups. All analyses were performed with GraphPad InStat
software (San Diego, USA). All the statements of significance
were based on a probability level of p<0.05.

Results
Gross lesions and relative weight of kidney and liver
Macroscopic examination of rats fed with OTA or Endosulfan
or their combination for 30 days, showed focal areas on
liver surface. Liver was slightly pale and molted. There was a
significant reduction in relative weight of kidney and liver in
Group-I (OTA-treated) and Group-III (combination of OTA and
endosulfan diet) as compared to those of controls (Table 1).
The reduction was highly significant in Group-III.

Ochratoxin A (OTA) treatment
Kidney
As compared to controls (Figure 1), the proximal convoluted
tubules in OTA-treated animals showed degenerative changes
along with the presence of pinkish homogenous mass in the
lumen (Figure 2). Some of the tubules showed pyknotic and
karyorrhectic nuclei. Vascular congestion was observed both
in cortex and medulla. Several glomeruli showed widening
of Bowman's space with deposition of proteinaceous mass.
Tubular lumen was found to be obliterated due to degenera-
tion and swelling of lining epithelial cells.

Figure 1. Kidney section showing normal glomeruli and
proximal and distal convoluted tubules H&E×100.
Liver
Rats fed on toxin-free diet showed normal liver architecture (Figure 3). In comparison, hepatocytes in rats fed with OTA (Group-I) showed degenerative and necrotic changes with granular cytoplasm (Figure 4). Cytoplasm in most of the hepatocytes was pale or less stained. Hepatic sinusoids along with central and portal veins showed mild to moderate congestion. Mild to moderate fatty changes were observed in hepatocytes of centrilobular area (Figure 4).

Endosulfan treatment

Kidney
Proximal convoluted tubules in group–II, revealed degeneration and necrosis of the lining cells. The tubular lumen showed presence of detached necrosed pinkish mass (Figure 5). Inter-tubular blood vessels were mildly congested.

Liver
Hepatocytes in endosulfan-toxicated group-II rats, showed mild degeneration with granular cytoplasm in comparison to OTA alone (Figure 6).

Endosulfan+OTA combination treatment

Kidney
In group-III, proximal convoluted tubules exhibited degenerative changes with pinkish granules in the cytoplasm and vacuolations in the lining of epithelial cells (Figure 7). The swelling of the cells resulted into the obstruction of the lumen. Some of the glomeruli showed presence of pinkish homogenous

Table 1. Effect of OTA and Endosulfan (ES) administered alone and in combination on relative weight of liver and kidney of adult male rats.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Organ weight (gm) expressed as mean± SE (n=10)</th>
<th>Control</th>
<th>OTA (ES)</th>
<th>Combination (OTA+ES)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td>3.93±0.24b</td>
<td>3.27±0.66a</td>
<td>3.57±0.18b</td>
<td>3.12±0.11a</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>1.03±0.07b</td>
<td>0.79±0.99a</td>
<td>0.86±0.04b</td>
<td>0.70±0.064</td>
</tr>
</tbody>
</table>

Mean bearing at least one common superscript do not differ significantly between groups.

Figure 2. OTA-treated kidney: Marked necrotic and degenerative changes in proximal convoluted tubules. Note the detachment of necrosed lining cells H&E×200.

Figure 4. OTA-treated liver: Marked fatty changes and degenerative changes in hepatic parenchyma H&E×200.

Figure 3. Liver section showing normal architectural arrangement of hepatic cords. The hepatocytes also appear normal with centrally placed nucleus and eosinophilic cytoplasm H&E×100.
proteinaceous mass and vacuoles in the capillary tuft with increased Bowman's space (Figure 7). The inter-tubular blood vessels were occasionally congested.

Liver

Hepatic sinusoids and portal veins in group-III animals, showed mild to moderate vascular congestion. Swelling with fine granules in the cytoplasm and occasional double nuclei was observed in hepatocytes. Hepatic lobules revealed degeneration with presence of small to large fatty vacuoles in hepatocytes pushing the nucleus towards periphery (Figure 8). These fatty changes were mostly confined around the central vein. At places mononuclear cell reaction was noticed in the centre of the portal lobules around the portal triad. Focal areas of necrosis with isolated hepatocytes were seen in hepatic parenchyma at few places (Figure 8).

Discussion

Gross lesion observed were swollen and slightly mottled liver and slightly pale kidneys. The relative weight to these organs was also found to be decreased. The changes in liver and kidneys were comparatively more intense in combination group as compared to OTA or Endosulfan alone. These observations were in accordance with the findings of earlier
worker [29-31]. The absolute and relative organ weight of liver and kidney were in agreement with the observations of [32] in rats and [33] in chicks administered with Endosulfan. Contrary to our observations on organ weights, earlier workers [34-36] reported increased organ weight of liver and kidneys. These discrepancies could be due to many factors such as age, sex and strain of animals, the influence of the vehicle or difference in the administration regimen. OTA and Endosulfan alone caused pale discoloration of liver and kidneys, increased fragility of liver, which could be attributed to degenerative changes as detected on histopathological examination. OTA and Endosulfan are known to be hepatotoxic and nephrotoxic. These effects have been seen in our experimental animal rat. The changes in liver were of degenerative in nature including fatty changes and vascular congestion. Similar changes have also been reported by earlier workers in different species of animals i.e., rat [29,30,36], rabbits [31,37-39] and poultry [40]. The degenerative and vascular changes observed in kidney tubules and renal corpuscles were in consensus with the findings of earlier workers [29,36,40,41]. The gross or microscopic changes observed in the present study may be classified as mild, probably due to low dose of ochratoxin-A or endosulfan. The histopathological changes in kidneys explain, at least in parts, the functional alterations (increased level of creatinine and blood urea nitrogen) recorded in serum analysis of these experimental rats in a previous study [42]. From the present study it was concluded that OTA and Endosulfan are potentially toxic to liver and kidney in combination as compared to their toxic effects alone.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

<table>
<thead>
<tr>
<th>Authors' contributions</th>
<th>SNK</th>
<th>BB</th>
<th>AGT</th>
<th>KPS</th>
<th>RS</th>
<th>AKJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research concept and design</td>
<td>✓</td>
<td>--</td>
<td>✓</td>
<td>--</td>
<td>✓</td>
<td>--</td>
</tr>
<tr>
<td>Collection and/or assembly of data</td>
<td>✓</td>
<td>--</td>
<td>--</td>
<td>✓</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Data analysis and interpretation</td>
<td>✓</td>
<td>--</td>
<td>--</td>
<td>✓</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Writing the article</td>
<td>✓</td>
<td>✓</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Critical revision of the article</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>✓</td>
</tr>
<tr>
<td>Final approval of article</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>✓</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>--</td>
<td>--</td>
<td>✓</td>
<td>✓</td>
<td>--</td>
<td>✓</td>
</tr>
</tbody>
</table>

Acknowledgement

The researchers would like to acknowledge the help extended by In-charge, Division of Pathology, Indian Veterinary Research Institute.

Publication history

Editor: Lingyan Wang, Oregon Health & Science University, Portland.
EIC: Gaetano Giuseppe Magro, University of Catania, Italy.
Received: 29-Sep-2015 Final Revised: 03-Nov-2015
Accepted: 13-Nov-2015 Published: 23-Nov-2015

References

2. Omnipresent Poison. | Website
21. Pfohl-Leszkowicz A and Manderville RA. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. Mol Nutr Food Res. 5
42. S. N. Kumar, A. G. Telang, K. P. Singh, B. Bastia and A. K. Jain. Toxic Manifestation of Endosulfan and Ochratoxin- A in Adult Male Rats. MOJ Toxicol. 2015; 1:00012. | PDF

Citation:
http://dx.doi.org/10.7243/2055-091X-2-22