Dietary cancer risk from conditional cancerogens (tumor promoters) in produce of livestock fed on species of spurge (Euphorbiaceae)

IV. Toxicologic and pathophysiologic observations in lactating goats and their suckling kids fed on the irritant herbs Euphorbia nubica and Euphorbia helioscopia: an etiologic model for investigations on the putative risk of cancer by consumption of food polluted with tumor promoters

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Abstract The feeding of lactating goats on usual green fodder, contaminated with Euphorbia helioscopia or E. nubica, results in poisoning of the dams as well as their suckling kids. General signs of toxicity were emaciation, depression, shedding of body hair, arching of back, and possible death. Post-mortem changes of dams and dead suckling kids included congestion and hemorrhage in cardiac muscle, lung, liver, and kidneys. Blood analyses of goats exposed to these contaminants showed an increased level of serum alanine amino transferase compared to control samples, indicating cellular destruction in the liver. The latter was confirmed by histopathological changes in the organ which include severe congestion, necrosis, and degenerative changes. The goats also suffered from deterioration of renal function as indicated by increased blood urea nitrogen and creatinine levels. In histopathologic inspections of kidney, severe congestion, hemorrhage in the cortex and medulla, as well as necrosis of epithelial cells of kidney tubules were noticed. Considerable degenerative changes were also observed in heart and lung. The pathophysiological appearances indicate that by feeding on the Euphorbia species mentioned above, the goats are poisoned in a way similar to the case of E. peplus reported previously. Such intoxication most likely is due to irritant and hyperplasiogenic diterpene ester (DTE) toxins, usually present in the aerial parts of Euphorbia species and well known as tumor promoters in mouse skin. After ingestion of the toxic plant parts by the goats, the DTE toxins might be metabolized and thereby partially detoxified. Yet, at least in part, they may show up in the milk of the goats, as indicated by severe poisoning of their suckling kids. As discussed previously in lactating goats fed on fodder contaminated with E. peplus, tumor promoters of the DTE type may enter the human food chain via this source of milk. Such milk may be considered a valuable etiologic model for the investigation of economic, ecologic, and public health problems raised by human diet polluted with tumor promoters, i.e., conditional (non-genotoxic) cancerogens.

Key words Conditional (non-genotoxic) cancerogen · Contaminated fodder · Diterpene ester toxins · pathophysiologic observations · Euphorbia nubica · Euphorbia helioscopia · Goat milk, polluted · Human food chain · Life-style cancer · Risk of dietary cancer · Toxicologic observations · Tumor promoter, diterpene-ester type

Introduction

In preceding papers (Nawito et al. 1998; Zayed et al. 1998a) it was suggested that milk from lactating goats polluted with toxins of the DTE type possibly may be used as an etiologic model for investigations on the putative risk of cancer by ingestion of food contaminated with tumor promoters [i.e., conditional (non-genotoxic) cancerogens]. By feeding dams on some irritant species of spurge (Euphorbiaceae), for example, aerial parts of Euphorbia peplus, mixed with their usual green fodder,
general poisoning was noticed as indicated by loss of appetite, stiffness, and general weakness. Moreover, the milk of the dams fed on *E. peplus* caused poisoning in their suckling kids and even death with pathophysiological appearances similar to those in the mother goats. These results verified the hypothesis that the irritant DTE toxins occurring as certain *Euphorbia* factors of the well-known tumor-promoter type in parts of *E. peplus* that are eaten (Zayed et al. 1998b) may be responsible for the intoxications of both the dams and their milk-raised kids. The findings opened up new perspectives regarding a certain risk of life-style cancer caused by habitual consumption of goat milk from certain sources. Interestingly, for example, an increased incidence of esophageal cancer is prevalent in the littoral of the Caspian Sea where people habitually consume large amounts of goat milk (and sheep milk) (Hormozdari et al. 1975). The causes for this high incidence were investigated by the International Agency for Research on Cancer (WHO) to detect suspected solitary carcinogens in the milk consumed, such as e.g., nitrosamines (Hormozdari et al. 1975), possibly polluting goat milk in that region. Alternatively, our recent findings (Zayed et al. 1998a) suggest that the high incidence of esophageal cancer in the region mentioned above may be attributed to exposure of the initiation/promotion type: people are exposed to subcancerogenic doses of (genotoxic) solitary carcinogen(s), of whatever origin initiating target tissues. Consumption of milk polluted with tumor-promoting (conditional, non-genotoxic) cancrogenic DTE toxins may then elicit the tumors observed. In view of the economic, ecologic, and public health problems involved in consumption of milk polluted with tumor promoters, it appeared mandatory to try to confirm and establish the etiologic model proposed by investigation of the effects on lactating goats of fodder contaminated with other *Euphorbiaceae* species, such as the herbaceous and irritant *E. nubica* and *E. helioscopia* occurring in Egypt (Zayed et al. 1998a).

Throughout, the terms used regarding chemical carcinogenesis follow the definitions presented by Appel et al. (1990), see also Arco et al. (1995). The terminology of toxic *Euphorbia* factors and non-toxic *Euphorbia* substances used was defined by Sosath et al. (1988).

### Materials and methods

#### Plant material

*E. nubica* N.E.Br. plants were obtained from coastal regions at the Red Sea. *E. helioscopia* L. plants were collected at Fayum oasis from barseem (*Trifolium alexandrinum*) and fields of green fodder. Samples of the plants were identified by Professor Dr. N. El-Hadidi, Department of Botany, Faculty of Science, Cairo University, Egypt.

Aerial parts of each plant were air dried at room temperature and then milled. The powdered plant was prepared in boluses, each containing 3 g of plant powder, 30 g of corn flour, and 15 g of treacle mixed with 15 ml of water. Boluses were kept in a refrigerator till use.

#### Experimental animals (see also Nawito et al. 1998)

Healthy 3-year-old female lactating goats with their kids were used for these studies. Goats were of the Egyptian Baladi breed, weighing about 25 kg each. Animals were examined and treated if necessary against any external or internal parasites. They were also tested to ensure that they were free from brucellosis or mastitis. Goats were fed on barseem, and given water twice daily. Animals were kept in the Experimental Animal Farm of the Department of Animal Reproduction, National Research Center, Cairo, at Abu-Rawash, Giza.

#### Feeding experiments (for protocols employed, see Tables 1 and 2)

Within the groups of treated animals, each mother goat received a daily dose of 30 g of plant material mixed into its regular fodder. In the control groups, mother animals were fed on a normal diet only. Kids in both groups were only allowed to feed on their mothers’ milk. In the case of *E. nubica*, the experimental groups consisted of three mother goats with four kids as exposed animals and two mother goats with two kids as control animals (Table 1). Analogously, for *E. helioscopia*, three goats with four kids were exposed, whereas three goats with three kids made up the control group (Table 2). Feeding with contaminated fodder was conducted for 13 days followed by a 10-day recovery period in which only regular fodder was administered. On day 18 (also on day 23 in the case of *E. nubica*) the health status was registered. In the case of *E. helioscopia*, on day 23, some animals were killed for pathologic inspection.

#### Biochemical parameters (see Tables 3 and 4 in Results)

Blood samples from mother goats were taken during the feeding experiment. For comparison, blood samples were also taken before and after the feeding period. Blood was collected, samples for

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Feeding period (day)</th>
<th>Post-feeding period (day)</th>
<th>Health status on day</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Regular fodder</td>
<td>Mk</td>
<td>Mk</td>
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</tr>
<tr>
<td>(controls)</td>
<td>Mk</td>
<td>Mk</td>
<td>Mk</td>
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<tr>
<td>Contaminated fodder</td>
<td>Mkk</td>
<td>Mkk</td>
<td>Mkk</td>
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* Both died, autopsied

| M died, autopsied

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**Table 1** Synopsis of the feeding protocol of lactating goats using regular fodder and fodder with admixed aerial parts of *E. nubica*. Kids were fed only on their mothers’ milk. During the experimental period, blood samples from mothers were taken and analyzed for biochemical parameters. (M mother, k one kid, kk two kids)