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# Prevention of Fumonisin-induced Maternal and Developmental Toxicity in Rats by Certain Plant Extracts

**Mosaad A. Abdel-Wahhab,[1]* Azza M. Hassan,[2] Hany A. Amer[3] and Khayria M. Naguib[1]**

[1] Food Toxicology and Contaminants Department, National Research Centre, Dokki, Egypt
[2] Forensic Medicine & Toxicology Department, Faculty of Medicine for Girls, Al-Azhar University, Nasr City, Cairo, Egypt
[3] Animal Reproduction and Artificial Insemination Department, National Research Centre, Dokki, Egypt

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**In earlier work we have reported that garlic and cabbage extracts can protect laboratory animals from the toxic effects of different mycotoxins. Previous research demonstrated that fumonisin (FB) induced developmental effects in mice, rats and hamsters. The objectives of the present study were to utilize the pregnant rat as an *in vivo* model to compare the potential of garlic and cabbage seed extracts to prevent the developmental toxicity of FB and the effects of these extracts on sphingolipid metabolism in dam and foetus livers. Six treatment groups included a control group, a group fed on an FB-containing diet (150 mg $kg^{-1}$ feed) and groups treated orally with garlic or cabbage extracts (5 mg $kg^{-1}$ body wt.) with or without FB during gestation days 6–15. Evaluations of toxicity were performed on day 20. These include: maternal (mortality, body weight, feed intake and litter weight), developmental (embryonic resorption, foetal body weight, foetal soft-tissue anomalies and foetal skeletal examinations) and maternal and foetal sphingolipid metabolism. Fumonisin alone resulted in significant decreases in feed intake, body weight gain, litter weight, number of live foetuses and foetal body weight, whereas it increased significantly the number of resorbed foetuses and the number of skeletal malformations (30.4% for skull and 26.08% for sternebrae) and also increased the sphinganine/sphingosine (Sa/So) ratio in dam but not fetus livers. Garlic alone or plus FB was comparable to the control regarding all the tested parameters. On the other hand, cabbage seed extract alone or plus FB resulted in 10% maternal mortality and a decrease in maternal body weight and litter weight. It resulted in 4.65% skull malformations in foetuses but it was comparable to the control with regard to the other tested parameters. It could be concluded that both garlic and cabbage seed extracts have protective effects in pregnant rats. Moreover, garlic extract was found to have a greater protective effect than cabbage seed extract. Copyright © 2004 John Wiley & Sons, Ltd.**

## INTRODUCTION

Several species of *fusarium*, including *F. moniliforme*, *F. proliferatum* and *F. nygamai*, which are common contaminants of corn and corn-based diets worldwide, have been associated with a variety of diseases in animals and oesophageal cancer in humans (Penner *et al*., 1998). They produce a class of mycotoxins, the fumonisins, that contaminate staple food grains and are of danger to humans and animals alike. They have been shown to cause foetal syndromes in horses (known as equine leukoencephalomalacia) and in swine (known as porcine pulmonary oedema) (Voss *et al*., 1996). An entirely different class of carcinogenic mutations is caused by the fumonisins (George, 1994). In rats, both culture material of *F. moniliforme* and purified fumonisin $B_1$ ($FB_1$), the most common fumonisin found on corn, are hepatotoxic and nephrotoxic (Voss *et al*., 1995) and they have cancer-promoting activity (Haschek *et al*., 1992). It has been suggested that $FB_1$ may be involved in reproductive failure in pregnant sows, mice, rats and hamsters (Penner *et al*., 1998). Following a report of suggested induction of abortion in swine by $FB_1$, Floss *et al*. (1994a) were able to demonstrate increased foetal loss, tail and digital malformation and cleft palate in hamsters exposed to pure $FB_1$ or *F. moniliforme* culture extract. Gross *et al*. (1994) also reported maternal mortality, reduced maternal weight gains, maternal hepatotoxic effects (ascitis, increased alanine aminotransferase (ALT), hepatocellular necrosis, etc), increased embryonic resorptions, decreased number of live offspring, reduced offspring body weight and anatomical defects in live offspring, including cleft palate, hydrocephalus and ossification deficits involving phalanges, sternum and ribs following gastrointestinal exposure of female mice to aqueous extract of *F. moniliforme* culture materials.

Fumonisin $B_1$ is a specific inhibitor of sphinganine *N*-acyl-transferase, leading to the inhibition of sphingolipid biosynthesis and an increase in the sphinganine/sphingosine (Sa/So) ratio *in vitro* and in a variety of tissues *in vivo*. Sphingolipid ratios must be used as a diagnostic tool for assessing fumonisin exposure in rats (Reddy *et al*., 1996). It could be argued that $FB_1$ stimulates DNA synthesis and

* Correspondence to: M. A. Abdel-Wahhab, Mycotoxins Laboratory, National Research Centre, Dokki, Cairo, Egypt.
E-mail: Mosaad_Attia@yahoo.com

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acts as a mitogen by lowering the concentration of a growth-inhibiting sphingolipid (George, 1994).

In latter years it has been found in certain models that garlic preparations prevent tumour promotion (Dorant *et al*., 1993), cardiovascular diseases (Kleijnen *et al*., 1989), liver damage (Nakagawa *et al*., 1989) and aging (Moriguchi *et al*., 1994), which are considered to be associated with oxygen radical injury and lipid peroxidation. McCord (1994) reported that peroxidation is a major cause of the decrease in the nutritive value of fat and oil products. This reaction generates very reactive oxygen compounds, which in humans are responsible for causing or accelerating chronic disease states such as cardiovascular, neoplastic inflammatory and amyloidosis and aging. The antioxidant effects of garlic extracts and their components have been demonstrated and quantified by a variety of assays (Horie *et al*., 1989; Yamasaki *et al*., 1994). Ide *et al*. (1996) reported that garlic and its major organosulphur constituents have a scavenging effect on hydrogen peroxide and inhibit the chain oxidation induced by a hydrophilic radical initiator. Gazzani *et al*. (1998a,b) found that organosulphur components of some common diet vegetables, including garlic and cabbage, had a protective activity against rat liver microsome lipid peroxidation induced by $CCl_4$ and measured by malondialdehyde release. The objectives of the present study are to evaluate the maternal and developmental toxicity of fumonisin and the possible protective effects of garlic and cabbage seed extracts as antioxidant agents in pregnant rats.

## MATERIALS AND METHODS

### Chemicals

Fumonisin B (FB) standard was purchased from Sigma Chemical Co. (St Louis, MO) and other chemicals were of the highest purity commercially available.

### Fumonisin production

Fumonisin was produced through the fermentation of corn by *Fusarium moniliforme* strain MRC 826 as described by Riley *et al*. (1994). The fermented corn was autoclaved and ground to a powder, and the FB content was determined by HPLC according to Shaphard *et al*. (1990). The corn powder was incorporated into the basal diet to provide the desired level of 150 mg $kg^{-1}$. The diet containing FB was analysed and the presence of FB was confirmed by HPLC.

### Preparation of garlic and cabbage extracts

Fresh garlic bulbs and cabbage seeds were purchased from the local market and were extracted with 95% ethanol in a Warring blender at room temperature as described previously by Fan and Chen (1999). The oil-soluble extracts (diallyl sulphide, diallyl disulphide, dipropyl sulphide and dipropyl disulphide) of both garlic and cabbage seeds were used in our study.

### Experimental animals

Three-month-old sexually mature virgin Sprague-Dawley female and male rats (200–210 g, purchased from an animal house colony, Giza, Egypt) were maintained on a standard laboratory diet (protein, 16.04%; fat, 3.63%; fibre, 4.1%, metabolic energy, 2887 $kcal^{-1}$) and water *ad libitum* at the Animal House Laboratory (AHL), National Research Center, Dokki, Cairo, Egypt. A total of 60 pregnant rats grouped into six treatment groups were used in this study (each group consisted of ten animals). After an acclimatization period of 1 week, females were paired with males overnight in filter-top polycarbonate cages housed in a temperature-controlled and artificially illuminated room (12-h dark/light cycle) free from any source of chemical contamination. Successful mating was determined by the presence of sperm in the vaginal smears and was designated as day 0 of pregnancy.

### Experimental design

The pregnant females were distributed into six treatment groups as follows: (1) untreated control; (2) FB alone (150 mg $kg^{-1}$ diet); (3) orally treated with garlic extract (5 mg $kg^{-1}$ body wt.); (4) orally treated with cabbage extract (5 mg $kg^{-1}$ body wt.); (5) on FB-containing diet and orally treated with garlic extract; and (6) on FB-containing diet and orally treated with cabbage extract. The pregnant females were housed separately in filter-top polycarbonate cages at the AHL and were maintained on these treatments throughout days 6–15 of gestation. Maternal body weight and feed intake were recorded daily.

### Assessment of developmental toxicity

Dams were killed on day 20 of gestation, uteri were removed and litters were weighed. The uterine horns were exposed and the numbers of implants, resorptions and live foetuses were counted. Live foetuses were removed from the uterus, blotted dry, weighed and examined for gross malformations. Dams and foetuses were evaluated further for various maternal, developmental and biochemical parameters.

One-third of the foetuses were immersed in Bouin's solution for assessment of internal soft-tissue anomalies using Wilson's free-hand razor-blade technique (Wilson, 1965). Another one-third of the fetuses were placed in 95% ethanol followed by alizarin red staining for evaluation of skeletal anomalies as described by Manson and Kang (1982).

### Hepatic sphinganine/sphingosine (Sa/So) ratio

To determine if the hepatotoxic effects of fumonisin in the dam correlated with disruption of sphingolipid metabolism, the ratio of free sphinganine/sphingosine (Sa/So) was determined in livers from five dams and a pool of the remainding one-third of the foetuses from each treatment group using the HPLC method of Riley *et al*. (1994). In the foetal liver, the Sa/So ratio was used as a biomarker of exposure of the offspring to FB.

### Statistical analysis

All data were analysed statistically using the General Linear Models Procedure of the Statistical Analysis System (SAS Institute, 1982). The significance of the differences among treatment groups was determined by the Waller–Duncan *k*-ratio (Waller and Duncan, 1969). All