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## Short Communication

# Sterigmatocystin toxicosis studied in rats

SHAMSIA BANU AND E.R.B. SHANMUGASUNDARAM

University Biochemical Laboratories, University of Madras, Guindy Campus, Madras 600 025.

**Abstract**

Sterigmatocystin toxicosis in rats was found to produce necrosis and hepatocytomegaly in liver and alteration in the levels of transaminases, gamma glutamyl transferase and derangement in carbohydrate metabolism. The possible mode of action of sterigmatocystin might be due to neoplastic condition which is related to these biochemical changes

**Key words:** Sterigmatocystin toxicosis, carbohydrate metabolism, neoplastic conditions.

## 1. Introduction

Sterigmatocystin - a metabolite of the mold *Aspergillus versicolor* - is a biogenetic precursor of Aflatoxin $B_1$ and reported to be a carcinogen for the rat[1]. Subcutaneous administration of sterigmatocystin to rats has been reported to produce hepatoma and local sarcoma whereas oral administration induced hepatocellular carcinoma[2,3]. Recent trends on histochemical studies on hepatocarcinogenesis revealed that the observed changes in the liver cells, along with glycogen storage and increased activity of gamma-glutamyl transpeptidase in the tissues may serve as useful markers in the detection and identification of pre-neoplastic liver cells[4]. In rats increased gamma-glutamyl transferase activity has been demonstrated in hyper-plastic foci and nodules in Aflatoxin $B_1$-induced hepatocellular carcinoma[5]. Hence correlation between the biochemical and histological changes in liver tissue in rats treated with the mycotoxin sterigmatocystin at a level of 0.5 mg/kg body weight for a period of 55 weeks has been made in the present studies to explain the neoplastic action of the mycotoxin.

## 2. Materials and methods

All chemicals used are of analytical grade.

A strain of *Aspergillus versicolor* isolated in our laboratory from contaminated feed was used. It was grown in 1 litre of medium having the composition as used by Rabie *et al*[6]. After 21 days of growth, sterigmatocystin was isolated from the cultures and purified by the method of Vorster[7] with slight modifications. The purified compound was compared with an authentic sample of sterigmatocystin, a gift sample given by Dr. Thiel, M.R.C., South Africa.

Weaning albino rats of Wistar strain were divided into two groups. Group-I was injected with 1% sterilized gelatin and served as the control while group-II animals were injected intraperitoneally with 0.5 mg/kg body weight of sterigmatocystin dispersed in 1% gelatin

every alternate day for a period of 55 weeks. Both the groups of animals were maintained along with controls under identical conditions throughout the period of the experiment.

The animals were fasted overnight and kept under ether anaesthesia. The blood samples were collected by cardiac puncture. The liver was removed and homogenised to get a 20% homogenate in 0.5 M sucrose solution using *Potter Elevehjem* homogeniser. The homogenate was contrifuged at 45,000 g for 60 minutes at 2° C and the clear supernatent (post-mitochondrial fraction) was used for various enzyme estimations. The sera and liver samples were kept in an icebath and assayed for various constituents within 12 hours.

*Histology:* A piece of liver from each animal used for biochemical determinations was fixed in neutral 10% formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin.

*Determinations:* Blood glucose was estimated by the modified method of Sasaki *et al*[8], and liver glycogen was estimated by the method of Morales *et al*[9]. Protein contents in sera and liver homogenate was determined by the Lowry *et al* method[10], using bovine serum albumin as the standard. Alanine transminase was assayed according to the method of Reitman and Frankel[11]. Tissue gamma-glutamyl transferase was assayed according to the method of Orlowski and Meister[12] and serum gamma-glutamyl transferase was assayed following the method of Rosalki and Tarlow[13].

The levels of glycogen synthetase, hexokinase, glycogen phosphorylase and lactate dehydrogenase were assayed following the methods of Leloir and Goldemberg[14], Brankstrup *et al*[15], Cornblath *et al*[16] and King[17] respectively. All enzyme activities determined in this study were expressed in standard units. One unit (U) of enzyme is defined as the amount that catalyses the transformation of 1 mole of substrate per minute under the described assay conditions.

## 3. Results

The body weight of rats treated with sterigmatocystin initially decreased slightly; later there was normal growth.

Histochemical changes in liver cell showed necrosis and areas of focal lymphocytic infiltration along with periportal fabrosis wherein there was definite evidence of hepatocytomegaly with prominent nucleolus. Sometimes more than one nucleus was seen indicating pre-neoplastic stage. The nuclear chromatin was coarse and distribution was not uniform (figs. 1 and 2).

Changes in biochemical parameters studied are given in Table I. During toxicosis blood glucose gets increased significantly ($p<0.001$) with a decrease in liver glycogen and with a concomitant decrease in glycogen synthetase, lactate dehydrogenase and hexokinase activity and an increase in glycogen phosphorylase. The liver protein got significantly increased, while the serum alanine and aspartate transaminases also increased significantly. There is no significant change in the serum protein values. The lower amount of the transaminases in liver (Table I) indicates that the enzymes have diffused into the serum. Gamma-glutamyl transferase is an enzyme used to diagnise pre-neoplastic cells. Its activity increases significantly in both serum as well as liver.

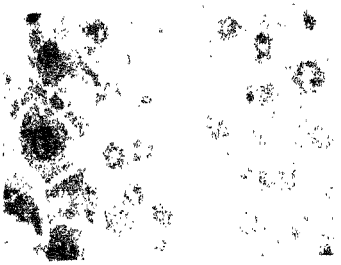

FIG. 1. Section of liver from rat injected with sterigmatocystin Haematoxylin-Eosin×400 (Hepatocytomegaly)

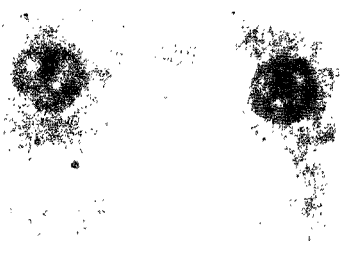

FIG. 2. Section of liver from rat injected with sterigmatocystin Haematoxylin-Eosin×400 oil immersion (Appearance of double nucleoli).

**Table I**

**Effect of sterigmatocystin on alanine transaminase, asparate transaminase and gamma-glutamyl transferase concentrations of rat serum and liver, blood sugar level and glycogen, glycogen synthetase, hexokinase and glycogen phosphorylase of rat liver[a]**

| | | Control | Toxin treated | P. value |
|---|---|---|---|---|
| Alanine transaminase | liver[b] | 3.58 ± 0.2 | 3.21 ± 0.10 | 0.01 |
| | serum[c] | 9.98 ± 0.18 | 12.79 ± 0.23 | 0.001 |
| Aspartate transaminase | liver[b] | 4.01 ± 0.12 | 3.21 ± 0.02 | 0.001 |
| | serum[c] | 11.75 ± 0.11 | 19.21 ± 0.09 | 0.001 |
| Gamma-glutamyl transferase | liver[b] | 0.55 ± 0.04 | 11.21 ± 0.08 | 0.001 |
| | serum[c] | 11.53 ± 0.34 | 15.21 ± 1.09 | 0.05 |
| Glycogen[d] | | 93.6 ± 2.74 | 43.68 ± 2.41 | 0.001 |
| Glycogen synthetase[e] | | 7.28 ± 0.04 | 4.30 ± 0.03 | 0.001 |
| Hexokinase | | 2.01 ± 0.16 | 0.52 ± 0.04 | 0.001 |
| Glycogen phosphorylase | | 0.32 ± 0.02 | 0.6 ± 0.03 | 0.001 |
| Blood sugar[f] | | 62.53 ± 4.18 | 91.83 ± 1.14 | 0.001 |

a) Rats were given intraperitoneally sterigmatocystin (0.5 mg/kg body weight) and killed after a period of about 55 weeks. Control groups received 1% gelatin alone. The values are the means ± S.D. of 6-8 animals.

b) The liver enzymes are expressed as units/mg of protein.

c) The serum enzymes are expressed as units/ml of serum.

d) Glycogen contents were given in mg/g of wet liver.

e) Glycogen synthetase activity is expressed as $n$ mol/mg protein·min

f) Blood sugar is expressed as mg/dl.

## 4. Discussion

Sequential histologic and histochemical studies revealed that hyper-plastic and pre-neoplastic liver lesions appeared at 32 weeks after the inception of sterigmatocystin supplemented diet[18]. The reduced level of liver glycogen during toxicosis indicates a derangement in carbohydrate metabolism. Cameron *et al*[19] and Fiala *et al*[20] reported earlier that glycogen storage is affected during sterigmatocystin toxicity. Sterigmatocystin is a precursor of Aflatoxin $B_1$ and an appreciable decrease in hepatic glycogenesis *in vivo* and a lowered glucose tolerance were demonstrated in Aflatoxin $B_1$–treated chicks[21]. Histopathology reports show chromosomal aberrations and bi-nucleated cells and during such changes levels of protein and RNA have been reported to increase slightly[22]. Increased levels of transaminases in serum signifies the leakage of enzyme due to cell damage and the results of sterigmatocystin toxicosis are well correlated with the report of Aflatoxicoses where levels of GOT and GPT are also increased[23].

It is suggested that both serum gamma-glutamyl transferase and liver gamma-glutamyl transferase activity are increased in both primary and secondary liver tumours[24]. However, tumour tissues may themselves contribute to the high serum gamma-glutamyl transferase activity and this is independent of clinical stages of development of tumour and occurs even when the filling defects were not seen. Hence gamma-glutamyl transferase is a marker enzyme for neoplastic stage and hepatocytomegalic condition as seen from the pathomorphology of the liver of rats subjected to sterigmatocystin toxicosis.

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