J. Indian Inst. Sci., 64 (C), June 1983, Pp. 65–71

Changes in carbohydrate metabolism during patulin toxicosis studied in chicks

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Received on October 22, 1981; Revised on October 9, 1982.

Abstract

The mycotoxin patulin is found to interfere with the carbohydrate metabolism. Enhanced glycogenolysis, depletion of glycogen, decrease in rate of glycolysis, activation of HMP pathway and increased gluconeogenesis have been observed during patulin toxicosis.

Key words : Mycotoxin, patulin.

1. Introduction

Patulin is a mycotoxin produced by a number of organisms including *Penicillium patulum* which are reported as a common food contaminants[1]. It inhibits the growth of many organisms[2], is carcinogenic when administered subcutaneously to rats[3] and produces teratogenic effects on chick embryos[4]. The results presented in this paper concern with the effect of patulin on carbohydrate metabolism.

2. Materials and methods

Patulin was isolated and purified from the concentrated culture filtrate of *Penicillium patulum* according to the method of Scott and Kennedy[5].

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Thirty, a day-old white Leghorn chicks obtained from the Tamilnadu Poultry Research Station, Madras, were divided into two groups. One group of birds was orally fed with 100 mcg of isolated patulin every 48 hours, while the other group served as control. Both groups were fed with commercial chick diet and water was given *ad libitum*. At the end of 15th dose of administration the birds were fasted overnight and killed by a blow on the head. Liver, kidney and intestine were removed carefully for biochemical investigations.

All the estimations were carried out within 12 hours after sacrificing the animals. The homogenates of the tissues were kept in an ice bath at 0°C while in use. Total alkali extractable carbohydrate and glycogen were extracted and estimated by the method of Morales *et al*[6], while tissue lactate was estimated by the method of Baker and Summerson[7] and pyruvate by the procedure of Friedeman and Haugen[8]. Glycogen phosphorylase was assayed by the method of Cornblath[9], hexokinase by the method of Branstrup *et al*[10], aldolase by the procedure described by King[11], G6PD by the method of Ells and Kirkman[12], FDPase by the method of Gancedo and Gancedo[13] and G6Pase by the method of Koide and Oda[14]. For enzyme assays the tissues were homogenised in *tris*-HCl buffer, pH 7·5 (0·01 M) at 4° C.

3. Results

Table I gives the levels of total alkali extractable carbohydrate, glycogen, lactate and pyruvate in liver, kidney and intestinal tissues from control and patulin-treated chicks. From the table it can be seen that the total alkali extractable carbohydrate levels is seen reduced in experimental birds by 70·5 per cent in liver, 64·0 per cent in kidney and 63·0 per cent in intestine while the glycogen content is also decreased by 67·4 per cent, 49·0 per cent, 57·5 per cent in liver, kidney and intestine respectively. The liver lactate level alone is increased by 48 per cent in the case of patulin-administered chicks, whereas there is no change in the pyruvate levels.

Figures 1–6 represent graphically the levels of glycogen phosphorylase, hexokinase, glucose-6-phosphate dehydrogenase, aldolase, glucose-6-phosphatase and fructose-1, 6-diphosphatase in liver, kidney and intestine of chicks fed with patulin as compared with control chicks. From fig. 1, it can be seen that glycogen phosphorylase activity was increased in liver (52 per cent), kidney (51 per cent) and intestine (35 per cent) of chicks fed with patulin when compared with control chicks.

The decrease in the level of hexokinase observed in fig. 2 was 42, 45 and 50 per cent in liver, kidney and intestine respectively. The enzyme level of G6PD (fig. 3) had increased in all tissues studied—in liver (28 per cent), kidney (15 per cent) and intestine (50 per cent) while aldolase activity (fig. 4) showed marked reduction—liver (45 per cent), kidney (28 per cent) and intestine (62 per cent). Elevated levels of G6Pase and FDPase (figs. 5 and 6) were observed in patulin-treated chicks.

Table I

Total alkali extractable carbohydrate, glycogen, lactate and pyruvate content of liver, kidney and intestine of control and patulin-administered chicks (15 doses of 100 mcg of patulin on alternate days)

	Total alk. ext. carbohydrate		Glycogen		Lactate		Pyruvate	
	Control	Test	Control	Test	Control	Test	Control	Test
Liver	78·03 ± 8·02	23·42 ± 2·86*	30·68 ± 1·68	9·78 ± 0·82*	5·82 ± 0·38	11·45 ± 1·26**	0·21 ± 0·02	0·28 ± 0·03
Kidney	23·35 ± 2·06	8·42 ± 0·86*	6·76 ± 0·56	3·48 ± 0·26*	11·28 ± 0·96	12 14 ± 1·02	0·26 ± 0·03	0·32 ± 0·02
Intestine	24·46 ± 2·58	9·08 ± 1·12*	9·32 ± 0·85	3·96 ± 0·42*	2·48 ± 0·32	3·08 ± 0·30	0·16 ± 0·02	0·19 ± 0·01

Values are expressed as mg/g fresh tissue (mean ± S.D.).
*$p < 0·001$; **$P < 0·01$.

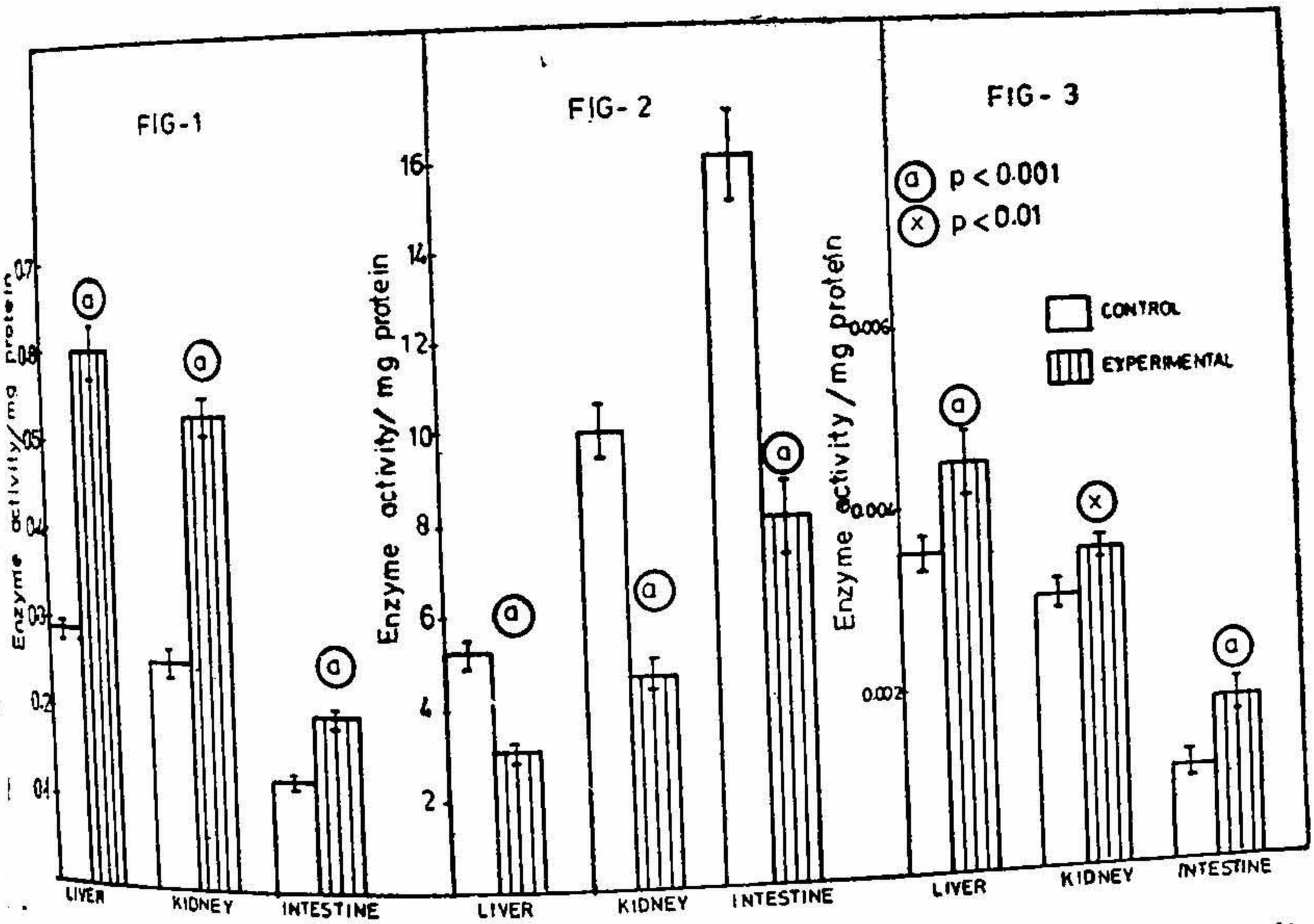

FIG. 1. Activity of glycogen phosphorylase. Activity is expressed as micro moles of Pi-liberated/ mg protein.

FIG. 2. Activity of hexokinase. Activity is expressed as micro moles of G-6-P formed/mg protein.

FIG. 3. Activity of G6PD. Activity is expressed as units/mg protein.

4. Discussion

The results indicate that the total carbohydrate and glycogen levels in liver, kidney and intestine were reduced drastically during patulin toxicosis. Shank and Wogan found a similar significant decrease in hepatic glycogen content after five successive doses of aflotoxin B_1 administration to ducklings. Shankaran *et al*[16] suggested that the aflotoxin B_1-induced glycogen depletion in chick liver could be due to an impairment of the glycogen synthetic mechanism. Marked reduction of hepatic glycogen accompanied by an increase of serum glucose was observed in mice treated with cycloheximide[17]. Ueno *et al*[18] stated that incorporation of ^{14}C-glucose into liver glycogen was found to be suppressed during such toxicoses. Madiyalakan and Shanmugasundaram showed an elevation in blood glucose and a reduction in the rate of incorporation of ^{14}C glucose into liver and kidney glycogen of mice treated with patulin suggesting suppression in the transport of glucose.

Our findings suggest that patulin-induced depletion of glycogen in chicks could be attributed to the inhibition of glycogenesis as evidenced by decreased hexokinase activity (fig. 2) as well as by the acceleration of glycolysis, and as seen by elevated glycogenphosphorylase activity (fig. 1).

The observed elevation in lactate levels may be due to defective glycolysis resulting in anaerobiosis. An earlier report had stated that patulin inhibits aerobic respiration in guinea pig kidney slices and brain homogenates[20]. This may lead to an unfavourable NAD/NADH ratio which could favour the conversion of pyruvate produced by glycolysis to lactate. Suzuki *et al*[21] reported a similar increase in liver lactate level accompanid by depletion of hepatic glycogen in ochratoxin A-treated rats.

The results obtained in the present investigation indicate a significant disturbance in the glucose metabolism. The increase in glycogen phosphorylase, a key enzyme of glucose metabolism suggested that the glycogen reserve was utilised during toxicosis. Hence the observed increase in glycogen phosphorylase could very well be correlated with the drastic reduction in glycogen content of patulin-treated chicks. An earlier study in our laboratory indicated that patulin administration to mice led to the activation of phosphorylase kinase which in turn leads to increased conversion of inactive form of the enzyme to the active form in liver tissue[22].

The decreased glycogen levels may be an outcome of a decreased glycogen synthesis as a result of decreased glucose-6-phosphate in the system which in turn results from the decreased hexokinase activity (fig. 2). Another possibility for the observed reduction of hexokinase activity is that patulin might have interacted with the -SH groups of hexokinase[23].

The reduction in aldolase activity (fig. 4) could be attributed to the interaction of patulin with –SH groups. Covalent interaction of patulin with amino groups

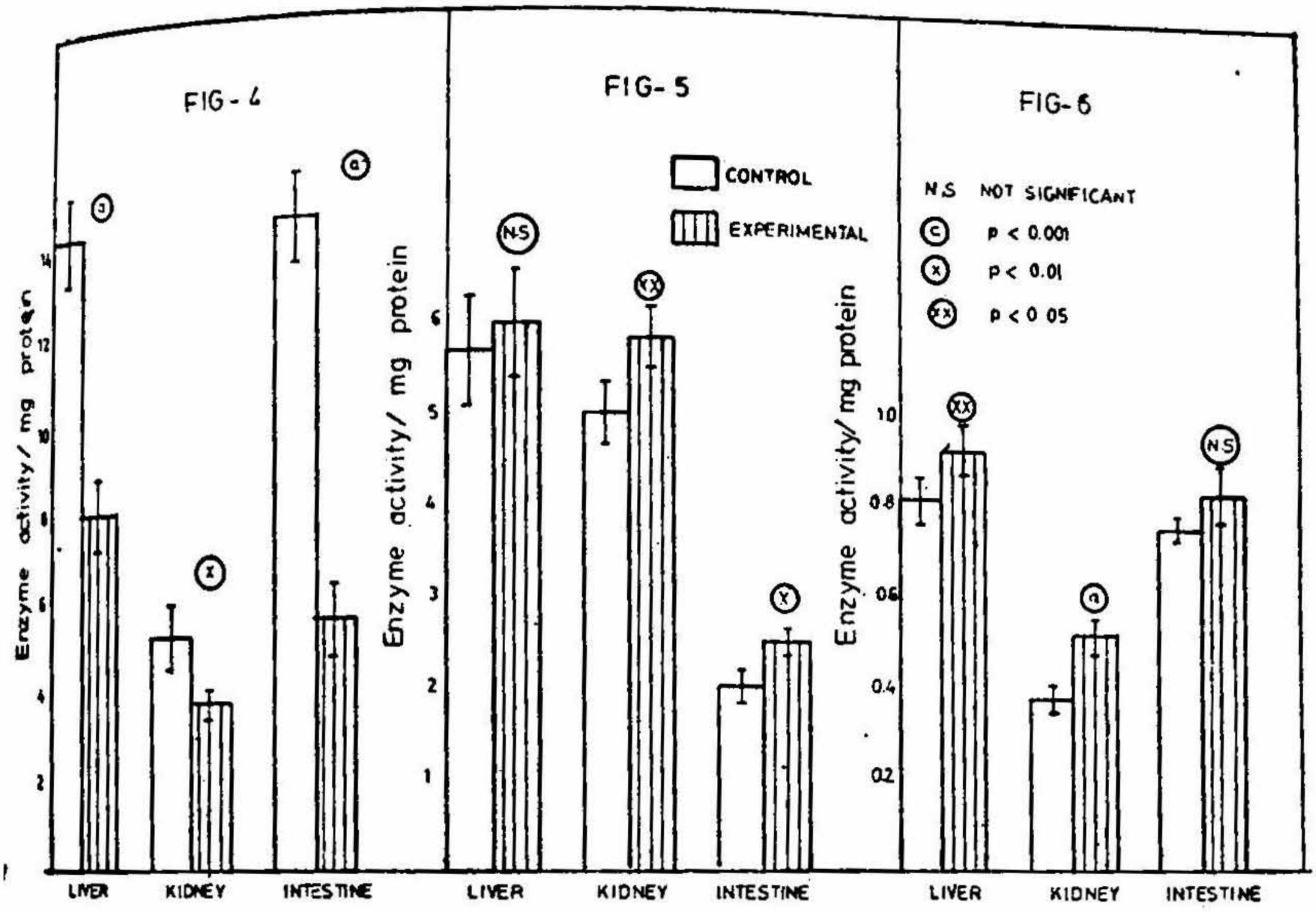

FIG. 4. Activity of aldolase. Activity is expressed as micromoles of glyceraldehyde formed/mg protein
FIG. 5. Activity of G6Pase. Activity is expressed as micro moles of Pi-liberated/mg protein.
FIG. 6. Activity of FDPase. Activity is expressed as micro moles of Pi-liberated/mg protein.

aldolase had also been demonstrated[24]. It is very interesting to see in our experiment that while the activity of aldolase is decreased, its substrate fructose-1, 6-diphosphate is acted upon by increased levels of FDPase (fig. 6), an important gluconeogenic enzyme. Increase in this enzyme is synonymous with increased gluconeogenic activity[25] and during patulin toxicosis such a situation may result.

The HMP shunt is activated by the observed increase in G6PD activity (fig. 3). Since glycogen synthesis is reduced and aldolase activity is also inhibited glucose-6-phosphate is channelled into the HMP pathway.

The overall picture of the derangement in glucose metabolism during patulin toxicosis as observed from our experiments may be summarised as: (i) enhanced glycogenolysis and inhibition of glycogenesis, (ii) slackening of glycolysis, (iii) the shunting of phospho-hexoses towards HMP pathway and (iv) increased gluconeogenesis.

Acknowledgement

HD thanks the University Grants Commission for financial assistance.

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