RESPONSES OF THE RAT'S LIVER AND SERUM GLUTAMATE AND
ISOCITRATE DEHYDROGENASES AND ORNITHINE CARBOMYL-
TRANSFERASE TO A HAEMAGGLUTININ FROM LIMA BEAN
(*Phaseolus lunatus* Linn.)

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ABSTRACT

The responses of liver and serum glutamate dehydrogenase (GDH; EC 1.4.1.2.), isocitrate dehydrogenase (ICDH; EC 1.1.1.42) and the urea cycle enzyme, ornithine carbamoyltransferase (OCT; EC 2.1.3.3) to dietary lima bean haemagglutinin (lectin) were assessed using a total of 54 growing rats.

The GDH and ICDH activities in the liver were highly significantly ($P < 0.001$) elevated by dietary haemagglutinin. The activities correlated significantly ($P < 0.01$) with the haemagglutinin levels in a positive quadratic fashion as judged by their respective R^2 , coefficient of multiple determination of 0.91 and 0.95. Liver OCT activity was significantly ($P < 0.05$) depressed but correlated poorly with haemagglutinin levels.

Activities of the serum dehydrogenases were significantly ($P < 0.05$) altered by dietary haemagglutinin. Although serum OCT activity tended to increase, such increases were however not significant. Serum GDH and ICDH values correlated significantly ($P < 0.05$) with haemagglutinin levels with respective R^2 values of 0.71 and 0.67.

The veterinary implication is highlighted and some remedies suggested.

INTRODUCTION

It is well documented that many of the legumes which play an important part as components of the diet of a

large segment of the world's population possess little nutritive value and may in fact be toxic unless subjected to some form of heat treatment (Liener, 1969). The phytohaemagglutinins (lectins) are prominent among the endogenous toxic constituents of edible legumes. The deleterious effects accompanying the feeding of raw phytohaemagglutinin containing legumes have been reported (Liener, 1953; Jaffe and Vergalette, 1968; Aletor and Fetuga 1984 a & b). Deaths arising from the intraperitoneal (i.p) injection of known amounts of certain legume phytohaemagglutinins have similarly been reported (Manage *et al.*, 1972; Ikegwuonu and Bassir, 1977, Ologhobo and Fetuga 1983).

Among the physiopathological changes induced in animals administered castor bean (*Ricinus communis*), soyabean (*Glycine max*) and field bean (*Vicia faba*) haemagglutinin include increases in blood urea, glucose, bilirubin, transaminases and lactic dehydrogenase (Dirheimer *et al.* 1966).

Available literature indicate that in spite of the high protein productivity potential of lima bean which is

surpassed only by soyabean (Luse, 1975). information on the potential toxic effects of some of its naturally occurring components have remained comparatively scanty.

This report, therefore examines the effects of feeding one of such components on some enzyme systems in the rat; highlights the veterinary implications as well as suggests some remedies given the potentials of this legume in narrowing the dearth of protein supply.

MATERIALS AND METHODS

The lima beans used were obtained from local farms in Okpebho local government area of Bendel State where it ranks next to cowpea as the commonly grown and consumed pulses. The phytohaemagglutinin was extracted from the finely milled, defatted bean flour by the 4-step purification method of Huprikar and Sohoni (1965) with modifications suggested by Bassir and Ikegwonu (1975). After exhaustive dialysis for 36 hours the haemagglutinin solutions were concentrated for 18 hours at 4°C by vacuum dialysis. The pooled solutions were thereafter lyophilized in a centrifugal freeze dryer, weighed and stored at -12°C in sealed ampoules prior to use.

The haemagglutinating potency of the raw bean as well as the lyophilized extract was determined by the two-fold serial dilution technique of Kabat and Mayer (1961) using 0.25% trypaninated rabbit erythrocytes.

Animals and their management

54 male and female Wistar albino rats obtained from the pre-clinical rat

colony of the college of medicine, University of Ibadan were used in this study. The animals were weaned at 21 days, fed standard laboratory animal stock diet until they were 28 days old weighing (59.61 – 63.88g).

The composition of the basal diet reported elsewhere (Aletor and Fetuga 1984 a & b) was formulated to supply 16% crude protein on dry matter basis. The basal diet was screened for possible haemagglutinating activity, a step designed to avert any superimposition of the incorporated haemagglutinin. Haemagglutinin was incorporated into the experimental diets at the expense of corn starch at the following levels: 0.00 (control), 15.72, 31.72, 47.16, 62.88, 78.60, 94.32, 110.04 and 125.7mg per 100g diet which were multiples of the levels determined to be present in the raw bean. The rats were reared on the 9 diets for 21 days during which food and water were offered unrestricted.

Enzymatic studies in blood and liver.

The rats were starved overnight at the end of the experiment. All rats from each dietary group were stunned and bled by neck decapitation and the blood allowed to flow freely into 10ml centrifuge tubes surrounded by melting ice. The blood was refrigerated for about 4 – 6 hours after which the serum separated out clearly.

While the sera separation was being awaited, the livers were immediately excised and blotted dry of blood with filter paper. Depot fat, connective tissues as well as the inactive regions of liver were removed. Liver tissue (2g) was homogenized in 10ml. of ice-cold 0.1M. Tris-HCl buffer, pH 7.4 and then made up to

100ml. with same buffer to give 2% (w/v) homogenate.

Activities of liver and serum glutamate dehydrogenase (GDH; EC 1.4.1.2) and isocitrate dehydrogenase (ICDH; EC 1.1.1.42) were immediately assayed for by the colorimetric method of King (1967). Ornithine carbamoyltransferase (OCT; EC 2.1.3.3) was determined by method of Cerriotti and Gazzaniga (1969). Serum and liver enzyme activities were expressed in IU/litre and IU/g fresh weight of liver respectively.

The data were subjected to factorial analysis of variance and regressions using the IITA, IBM-computer SPSSH (1976 version).

RESULTS

The responses of liver glutamate dehydrogenase (GDH), isocitrate dehydrogenase (ICDH) and ornithine carbamoyltransferase (OCT) to dietary haemagglutinin are shown in figures 1 and 3.

Both the liver GDH and ICDH activity values were highly significantly ($P < 0.001$) elevated due to the levels of haemagglutinin fed. The activities also correlated significantly ($P < 0.01$) with the haemagglutinin levels in a positive quadratic fashion as judged by their respective R^2 , coefficient of multiple determination of 0.91 and 0.95. The liver OCT activity decreased

Fig. 1. Liver Dehydrogenases as Influenced by Dietary Haemagglutinin (lectin)

significantly ($p < 0.05$) although correlated poorly with the dietary haemagglutinin levels.

Serum GDH activity was significantly ($P < 0.05$) depressed by dietary haemagglutinin while the ICDH activity (figure 2) showed an initial decrease from about 46 IU/litre in the control to about 43 IU/litre (37°C) at about 50mg % dietary haemagglutinin before a sharp rise over the control. Regression analysis showed the relationship between the serum dehydrogenases (GDH, ICDH) activities and dietary haemagglutinin levels to be significant ($p < 0.05$) with respective R^2 values of 0.71 and 0.67. Serum OCT activity increased though non-signifi-

cantly. It also correlated poorly with the haemagglutinin levels fed as judged by the R^2 value of 0.41.

DISCUSSION

The present investigation contributes essentially as an extension to earlier knowledge, on the likely mode of action of toxic legume haemagglutinins. While most other studies with haemagglutinins had been by intraperitoneal (i.p.) injection, this was by feeding.

The results show that the activities of the serum and liver dehydrogenases (GDH and ICDH) and the urea cycle enzyme, OCT, are markedly

Fig. 2. Serum Dehydrogenases as affected by Dietary Haemagglutinin (lectin)

Fig. 3. Liver and Serum ornithine Carbamoyltransferase as influenced by Haemagglutinin (lectin).

altered even when fed small levels of lima bean haemagglutinin. Dirrheimer *et al.* (1966), Ikegwuonu and Bassir (1977), Ologhobo and Fetuga (1983) noted that values for urea, bilirubin transaminases and lactic dehydrogenase in blood of rats increased during acute i.p. injection of some legume haemagglutinins including lima bean.

The major mechanism of ammonia fixation is by the GDH reaction, a reversible reaction favouring the formation of glutamic acid from α -keto-glutarate and free ammonia. The activation of liver GDH in the present study is perhaps a consequence of increased catabolism of amino acids in the livers as was observed in an earlier study (Aletor and Fetuga, 1984b) when rats were fed raw haemagglutinin — containing lima bean. Increased

catabolism of amino acids makes the function of detoxifying excess ammonia imperative in which the least energetically expensive pathway is via the urea cycle (Freeland and Briggs, 1977). Therefore, the decreased activity of the liver urea cycle enzyme, OCT, by dietary haemagglutinin could prove fatal by impairing the optimal detoxification of excess ammonia to the less toxic urea. The slight, though non-significant rise in serum OCT may well be consequent upon an overwhelming need to deal with excess ammonia in the blood.

An increase in the metabolism of the 4-C-dicarboxylic acids in the Krebs Cycle is indicated by the elevation in the levels of serum and liver ICDH, in consonance with the suggestions of Cox and Davies (1970), that

ICDH plays a regulatory role in the Krebs Cycle.

Dietary lima bean haemagglutinin has produced the effects described in this paper. The main interest in elucidating the mode of action of the naturally occurring toxicants in legumes appears to be the latter's potentials in ameliorating the dearth in protein supply. Since monogastric animals often stray into plots of growing legume seeds which harbour these toxicants, the risk of similar effects as described for the rat on them should not be overlooked; hence conscious efforts should be made to develop new genetic stocks (through selection) with minimum levels of some or all of these legume-based toxicants. Such a step would fully compliment current efforts which mainly tend to emphasize yield, pest and/or disease resistance.

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