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Assessment of Congo Red-induced Liver Damage By Selected Serum Transaminase Levels

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The activities of L-alanine amino transferase and L-aspartate amino transferase in rabbit serum were assayed in the presence and absence of graded doses of congo red administered i.p. Blood was collected for a period of seven days from three groups of rabbits administered with 45 mg kg^{-1} body wt., 67.5 mg kg^{-1} body wt body and 90.0 mg kg^{-1} body wt., respectively and used for enzyme analysis. The result obtained showed that the two enzyme activities were inhibited significantly ($P< 0.05$) and the degree of inhibitions were dose-dependent with respect to the control group. Dose dependent inhibition of Congo red on the two transanminases was substantiated by varying percentage decreases of the enzyme activities as 37, 58.7 and 60.2% for the groups 1, 2 and 3, respectively for aspartate amino transferase. The same pattern of inhibition was observed for alanine amino transferase suggesting that both enzymes may be inhibited by similar mechanism.

Key words: Congo red, rabbit, serum, alanine amino transferase, aspartate amino transferase

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Introduction

Technological development nowadays has given rise to modern facilities and upliftment of social standards, which expose man to dangers and life hazards due to his involvement in various synthetic and chemical hazards. A large number of these chemicals are carcinogenic when the body comes in contact with them such as in occasions of environmental, occupational or even chemical exposure. (Robert ***et al.***, 1990). Cancer causing agents have been reported in small quantities in foods, drinks, medicines and even the air we breathe, soil and water at times (Lester, 1975).

Some of the various dyes we use in our daily chemical and essential products such as Congo red, direct brown 95 and direct brown 2 have been shown to be metabolised to active products like benzidine, which have been proved to be experimentally carcinogenic. (Rinde and Troll, 1975). The notion of the caranogenic potentials of some of these dyes emphasizes the need for analysis of their nature, structure, physical characteristics and exploiting theoretical mechanistic concepts to predict the possible mechanisms of their reactions in the body.

Congo red dye, which is most commonly, used shows some qualities which range from suitable colour, fastness to light, resistance to polar solvents, fixability to fabrics and other substances (Conn, 1977). Congo red is in the class of azo dyes with chromophore of aromatic ring being joined to the azo group. This means that it will exhibit the chemical reactions of aromatics and azo compounds. Congo red is used in biological staining to elucidate the microscopic structure and nature of animal tissues, clinical diagnosis of amyloid disease and nephrotic syndrome (Hood, 1984). Because benzidine is a toxic metabolite of congo red dye, which is also used, in therapeutic purposes, people are constantly coming into contact with it without any regard to the health hazards. Not much toxilogical assessment has been done on the metabolism of the dye especially with regards to important cytotoxic enzyme markers of cytotoxicity like the amino transferases nor within the dose ranges used in clinical diagnosis in Maiduguri and textile industries in the Northern Nigerian nor North east sub-region in particular. The study was therefore, done to evaluate the effect of congo red dye in rabbits using the selected serum transaminases as indices of evaluation of toxic damage with the hope that it will throw more light on the mechanism of toxicity.

Materials and Methods

Treatment of animals and drug administration

Twelve male rabbits of the same age (4 months) obtained from the Animal colony of Department of Biochemistry, University of Maiduguri, Borno State, Nigeria were stabilized on Pfizer feeds for three days in a well ventilated room with 12 h light and dark cycle. Thereafter, they were randomly distributed into 4 groups of 3 rabbits each and injected intraperitoneally with congo red dye stuff prepared in physiological saline. Groups 1, 2 and 3 were given 90 mg kg^{-1} body wt., 67.5 and 45 mg kg^{-1} body wt of congo red dye (i.p) respectively while the 4th group served as control and was given 0.5 ml physiological saline only.

All the rabbits were left for 24 h before the first test blood samples were collected from them intravenously. Blood samples were collected from each rabbit daily for 7 days and serum

clarified for assay of aspartate amino transferase and alanine amino transferase activity for assessment of congo red-induced cytotoxic damage.

Biochemical analyses

Assay of aspartate aminotransferase activity

The experimental procedure followed was described by Mohun and Cook (1959). Four dry test tubes were labeled for aspartate and another for the blank and put into water bath with temperature of about 37-38^{0}C. Aspartate transferase substrates of 1.0 ml was pipetted into 2 test tubes and left in a water bath for a minute followed by addition of 0.2 ml of serum and shaken gently to mix. 1 h after incubation, 2 drops of aniline citrate reagents was added to stop the reaction and then simultaneously, 0.2 ml of serum was added to the other test tube as blank. The reaction tubes were left to stand for 20 minutes after which the tubes were removed from the water bath. 10 of 1.0 ml of 0.4 N NaOH was added and read at 520 nm after 10 min. This was done and recorded for every weighted grouped rabbit on the day of the experiment. Standard calibration curve was prepared using standard pyruvate (10 mg ml^{-1}) and 1 ml dinitrophenyl hydrazine and 0.4 M NaOH from where the enzyme activities of each rabbit was calculated.

Assay of alanine amino transferase

The procedure followed in the assay of alanine amino transferase was described by Mohun and Cook (1959) and similar to the assay of aspartate amino transferase activity. Four dry tubes were labeled similarly and 1 ml of alanine transferase substrate pipetted into each of the tube and left in the water bath (37-38^{0}C) for a few minutes. To each of the test tubes, 0.2 ml of serum was added and shaken gently to mix. An hour later, 2 drops of aniline citrate reagent was added to stop the reaction. Simultaneously, 0.2 ml of serum was added to these 4th tube (blank) after the addition of aniline citrate reagent and left for 20 min.

One ml of dinitrophenyl hydrazine reagent was added to each of the tubes, left for 20 min and then removed from the water bath 1.0 ml of 0.4 N NaOH was added and the absorbance read at 520 nM after 10 minutes. Calibration curve was prepared as for the assay of aspartate amino transferase using standard pyruvate, dinitrophenyl hydrazine and 0.4 NaOH and used to calculate the enzyme activities.

Statistical comparison was done between the tests and set at ($P< 0.05$) (Mead and Curnow, 1983).

Results

The effect of varying degrees of congo red under ***in vivo*** conditions on the activities of aspartate amino transferase and alanine amino transferase are as presented on Tables 1 and 2, respectively.

Aspartate amino transferase activities of the group 1 (90.0 mg kg^{-1}), group 2 (67.5 mg kg^{-1} body wt. and group 3 (45.0 mg kg^{-1} body wt.) was 31.0±6.21 U L^{-1}, 32.14± 4 and 49.0±6.2 representing 60.2, 58.7, and 37.0% inhibitions, respectively relative to control enzyme activity of 77.8±3.21 U L^{-1}. The trend of control result showed that aspartate amino transferase was

Table 1: Effect of congo red dye on aspartate amino transferase activity

Treatment mg dye Kg^{-1} body wt.	Enzyme activity 1 U L^{-1}	Percentage decrease in activity %
Group 1 (90 mg Kg^{-1} body wt.)	31.00±6.2*	60.2
Group 2 (67.5 mg Kg^{-1} body wt.)	32.14±4.1*	58.7
Group 3 (45.0 mg Kg^{-1} body wt.)	49.00±6.2*	37
Group 4 Control	77.80±3.2*	-

(*P < 0.05 from control). Each value is the mean of 7 different values and their standard errors

Table 2: Effect of Congo Red On alanine amino transferase activities

Treatment (mg dye kg^{-1} body weight)	Enzyme activity (U L^{-1})	Percentage decrease in activity%
Group 1 (90mg kg^{-1} body wt.)	45.8±1.17*	63
Group 2 (67.5mg kg^{-1} body wt.)	59.7±2.02*	51.3
Group 3 (45.0mg kg^{-1} body wt.)	80.4±1.92*	34.0
Group 4 (Control)	122.6±2.0	-

Tabulated values are mean ± S.E. of 7 different values.(*P<0.05 from control)

inhibited in a dose - related pattern by congo red dye with the highest drug showing the highest inhibition. The percentage decrease in enzyme activity was significant (P<0.05) for all the groups compared with the control.

The result of the effect of congo red on the rabbit alanine amino transferase activities (Table 2) showed the same trend of results as observed in aspartate amino transferase assay. At the lowest concentration of 45 mg congo red kg^{-1} body wt. (group 3) the enzyme activity decreased to 34% and activity of 80.4±1.71 U L^{-1} body wt. and group 1 (90 mg kg^{-} body wt) showed enzyme activities of 59.7±2.02 U L^{-1} and 45.8±1.12 U L^{-1}, respectively representing 51.3 and 63% decrease in enzyme inhibitions, respectively. The decrease in enzyme activities at 45.0 mg kg^{-1} body wt congo red dye for alanine amino transferase and aspartate amino transferase was 34 and 37%, respectively which was not statistically significant (P<0.05). The result presented indicated that the dye inhibited the enzyme activities.

Discussion

The data presented in this work showed that intraperitoneal administration of congo red dye to 4 month old rabbits inhibited the activities of aspartate amino transferase and alanine amino transferase, two cytotoxic markers (Zimmermann, 1982) to varying degrees compared with the control elements in a dose dependent pattern. This observation is in agreement with earlier report (Obi ***et al.***, 1991) that the congo red inhibited the activities of serum alkaline phosphate and l- aspartate amino transferase in rats, chickens and rabbits, these effects being highest in chickens within the same doses.

These observations suggest that the dye was adequately absorbed to cause the varying inhibitory effects on the activities of the two enzymes, which are widely, distributed intracellular enzymes escaping into the blood during tissue injuries like cellular necrosis and cell proliferation. The activities of the enzymes are raised in occasions of tissue damages making them relevant diagnostic tools in clinical management of cytotoxicity. However, the result of this work is opposite to the above report since there was evidence of inhibition of the two enzymes. It is

possible that there were infect enzyme inductions, which were not apparent because of the inhibitory nature of congo red dye on the two transferases

This observation is supported by some reports that carcinogenic azo dyes such as congo red bind covalently to proteins from other organs apart from the liver and since the enzymes are proteins their activities are bound to be affected. Congo red is metabolically reduced to benzidine (Rinde and Troll 1975), which is an established bladder carcinogen. From the foregoing, it is conceivable that the inhibitory effect shown in this work may not be due to the dye perse but the metabolic product, benzidine that binds covalently to cellular macromolecules.

This affects their deleterious actions such as gene mutation, enzyme induction and enzyme inhibition (Gozukara ***et al.***, 1982). Enzyme inhibitory effect has been reported even though congo red is not meant for consumption. The dye can come in contact with people as it is used in detecting virility of Shigella (Daskaleros and Payne, 1985) and endoplasmic determination of the extent of easily ulcerated gastric cancer (Tatsuta ***et al.***, 1982).

The result of this study seems to confirm an earlier report (Obi and Maduka, 1998) that congo red reduced glutathione levels in rabbit and rat sera. Both reports are cytotoxic responses indicating challenges to liver by xenobiotic oxidants and hence call for caution in clinical and industrial applications of azo dyes.

Therefore, the inhibitory effect reported in this work calls for caution and deserves further investigation so that patients are not constantly exposed to this potential or other toxic agent.

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