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# Urinary Elimination and Metabolism of Nitrosamines in Different Dietary Protein Wistar Rat Models

Kuyooro, S.E.[1], Adebawo, F.G.[2], Okekearu P. C.[3], Maduagwu, E. N.[4]
[1](Department of Chemical Sciences, Bells University of Technology, Nigeria)
[2](Department of Wood and Paper Technology, Federal College of Forestry, Nigeria)
[3](Department of Biochemistry, University of Ibadan, Nigeria)
[4](Department of Biochemistry, University of Ibadan, Nigeria)

***Abstract :*** *It is necessary to establish comparatively whether or not there are differences in the mode of elimination of Nitrosodimethylamine (NDMA) and /or Aflatoxin B1 (AFB1) in urine as between different nutritional states of dietary protein. Four groups of albino wistar rats were fed protein free, normal protein repletion, low protein and high protein diets. NDMA was excreted in urine of rats placed on protein free diet at higher quantity (27.19%) than protein repleted rats (19.35%) and in low protein fed rats at a lower amount (22.82%) than in high protein fed rats (32.68%,) after NDMA was given. There was a lower urinary excretion of NDMA in protein free fed rats (22.18%) than in their protein repleted counterparts (33.77%) and low protein fed rats excreted more NDMA (27.14%) than those placed on high protein (11.79%), after a concurrent dose of NDMA and AFB1. It appears the elimination of NDMA is enhanced by protein free intake compared with normal protein repletion intake while it was reduced by low protein intake as compared to high protein intake. AFB1 increased the elimination of NDMA in normal protein repletion and low protein intakes whereas decreased NDMA elimination was observed for protein free and high protein intakes.*

***Keywords :*** *Protein Energy Malnutrition, Nitrosodimethylamine, Aflatoxin B1, Repletion, Dietary protein*

## I. Introduction

Protein Energy Malnutrition (PEM) is a spectrum of diseases arising from a deficiency of dietary protein especially in childhood [1]. Kwashiorkor, one form of PEM, results from both the quantitative and qualitative deficiency of dietary protein with adequate caloric intake. This disease in experimental animals presents an ideal and interesting model with which to study the role of nutritional status in the toxicity and carcinogenicity of environmental chemical. Chronic wasting in experimental animals such as occurs in PEM with its attendant structural changes in the liver and its clinical manifestations may complicate the whole pattern of drug disposition [2, 3].

Human beings are exposed daily, either deliberately or inadvertently, to a variety of chemical substances that abound in the environment; in food, water, air, soil, drugs, cosmestics and pesticides. Whereas many of these compounds are relatively safe, some of them are chronically toxic. The N-nitrosamines, a class of N-nitroso compound is a group of environmental contaminants which pose a serious hazard to public health. The emergence of N-nitrosamines as a new subject of toxicological investigation has arisen as a result of their toxic and high potency as animal carcinogens. It has been demonstrated that nitrosamines are formed easily from these precursors under the mildly acidic conditions of the stomach [4, 5]. Because of their various biological actions, for example, acute and chronic cellular injury [6, 7], carcinogenicity [8], teratogenicity and embryotoxicity [9], their elimination from body tissues and organs will have immense contribution to their toxicodynamics. The excretion of N-nitroso compounds in urine of humans [10, 11] and experimental animals has been reported [12, 13].

The aflatoxins, a group of mycotoxins known to be acutely toxic and highly carcinogenic to man, have been known as environmental contaminants for a long time [14, 15, 16]. Aflatoxins are established human hepatocarcinogens and are well known Hepatocellular Carcinoma (HCC) risk factors when present in foodstuffs. Environmental factors, exposure level, and duration of exposure as well as age, health and nutritional status of diet can influence the toxicity of aflatoxins. Aflatoxin B1 (AFBI) is the most hepatotoxic and carcinogenic of the aflatoxins. A wide range of dietary staples and agricultural products (maize, peanuts, groundnuts, and melon) are contaminated with a significant proportion of aflatoxins, particularly AFBI [17, 18, 19, 20]. Aflatoxin $B_1$ is excreted mainly through the bile. Faeces and urine are minor excretory routes for aflatoxin B1 [15].

The purpose of this study is to investigate the effect of different nutritional states of dietary protein on the mode of urinary elimination of dimethylnitrosamines in wistar rats and the possible synergistic effect of Aflatoxin B1 on the elimination of nitrosamines. Also the research is aimed to investigate the effect of nitrosamines on liver function.

## II. Materials And Methods

### *2.1 Chemicals and reagents*

Pure Dimetylnitrosamine [$(CH_3)_2NNO$, Mol. Wt. 74.08] were donated by Late Prof. R. Preussmann of Institute of Toxicology and Cancer Risk Factor, German Cancer Research Centre, Heldeberg, FRG. Aflatoxin [$C_{17}H_{12}O_6$, Mol. Wt. 312] were obtained from Sigma, USA.

### *2.2 Composition of the Montgomery and Dymock Reagent*

a. Solution A: Sulphanilic acid solution.

27.2g of potassium hydrogen sulphate and 3.46g of sulphanilic acid were dissolved in 1 litre distilled water.

b. Solution B: Naphthylethylenediamine (NEDA) solution.

0.4g N-(1-naphthyl) ethylenediamine dihydrohychloride was dissolved in 1 litre distilled water.

c. Solution C: 0.5% sodium carbonate solution.

0.5g sodium carbonate was dissolved in 100ml distilled water.

Except where stated, all chemicals used were of analytical grade (Analar).

### *2.3 Experimental Animals*

Healthy male albino (*Rattus norvegicus*) rats of the wistar strain, which weighed between 160g- 220g were used for the experiments. They were obtained from the animal house of Veterinary Physiology Department, University of Ibadan, where they had been fed commercial rat pellets *ad libitum* and given clean drinking water freely. They were housed in standard cages in the animal house, Department of Biochemistry, University of Ibadan and acclimatized for two weeks at room temperature ($27^oC$). They were fed normal rat chow and water *ad libitum.*

### *2.4 Experimental Diets*

Cornstarch was used as the source of carbohydrate, fish meal as the source of protein and vegetable oil as the source of oil in formulating the diets. Vitalyte salt vitamin mixture was the source of salts and vitamins. The experimental diets were prepared as specified by their composition described in Table 1. The constituents were mixed together thoroughly to achieve homogeneity in the preparation of the diets. They were then made into pellets to make eating easy for the animals. After which they were stored at $4^0C$ in large plastic containers and labeled until required.

**Table 1: Composition of the Different Dietary Protein Diets**

| Components | Experimental Diets | | | |
|---|---|---|---|---|
| | Protein Free | Protein Repletion | Low Protein | High Protein |
| Protein | _ | 27% | 8% | 65% |
| Carbohydrate | 85% | 59% | 78% | 23% |
| Oil | 11% | 10% | 10% | 8% |
| Salt and Vitamin Mixture | 4% | 4% | 4% | 4% |

### *2.4 Experimental Animal treatment*

Animals were divided into four groups and were fed the diets for 28days. Each group was further divided into two subgroups. A single oral dose of 21µg NDMA/kg were given to one subgroup and a concurrent oral dose of 21µgNDMA/kg and 0.022µg AFB1/kg given to the other subgroup of rats.

### *2.5 Collection Of Samples*

#### *2.5.1 Collection Of Urine*

The rats were placed in metabolic cages with separate facilities for collection of urine. The rats were given the experimental diets and drinking water *ad-libitum.* Urine was collected for a period of 24 hours before the oral administration of NDMA and/or AFB1. Another set of urine samples were collected for another 24 hours.

#### *2.5.2 Collection Of Serum*

NDMA and/or AFB1 were administered orally to rats. They were sacrificed 24 hours after and sera samples were obtained. Sera samples collected were preserved in a deep freezer or at $4^0C$ for a short time in a refrigerator. The activities of Alkaline phosphatase (ALP), Alanine amino transferase (ALT) and Aspartate amino transferase (AST), and bilirubin levels were estimated in the serum obtained from each animal.

### *2.6 Biochemical Analyses*

#### *2.6.1 Estimation of Nitrosamines in the urine*

The 24 hours urine was clarified by swirling with activated charcoal and filtered through Whatman No. 1 filter paper. To 1ml of the filtrate, 0.5ml of 0.5% sodium carbonate solution was added. The solution was then irradiated with UV light for 15mins after which 1.5ml each of the sulphanilic acid and N-1-naphthylethylenediamine dihydrochloride (NEDA) reagents were added [21]. The solutions were shaken and allowed to stand for 15 minutes. The resulting pink coloured solutions were measured at 550nm using a spectrophotometer.

#### *2.6.2 Estimation of AST, ALT, ALP and Bilirubin in the blood*

AST and ALT activities were determined following the principle described by Reitman and Frankel (1957). ALP activity was determined using the principle described by Englehardt *et al*., 1970.

### *2.7 Statistical Analysis*

Analysis was perfomed using Microsoft Excel statistical package. Results are expressed as mean ± S.D and comparison was done within each group.

## III. Results

**Table 2: The concentration of NDMA excreted in urine of different dietary protein rat models after the single oral dose of 16µg NDMA and a concurrent dose of 16µg NDMA and 0.022µg AFB1/kg.**

| | Conc. Of Nitrosamine (ug/ml) | | | |
|---|---|---|---|---|
| | PF | PR | LP | HP |
| NDMA | 0.144±0.11 | 1.745±0.04 | 1.278±0.07 | 1.820±0.52 |
| NDMA&AFB1 | 0.760±0.14 | 1.300±0.99 | 1.520±0.05 | 0.660±0.49 |

Values are mean ± Standard Deviation (SD)

**Table 3: % NDMA excreted in urine of different dietary protein rat models after the single oral dose of 16µg NDMA and a concurrent dose of 16µg NDMA and 0.022µg AFB1/kg.**

| | % Nitrosamine excreted | | | |
|---|---|---|---|---|
| | PF | PR | LP | HP |
| NDMA | 27.19 | 19.35 | 22.82 | 32.68 |
| NDMA&AFBI | 22.18 | 33.77 | 27.14 | 11.79 |

**Table 4: Activities of some enzymes and biochemical parameters in rats fed a protein free and those that had undergone protein repletion (64%) following a single oral dose of 16µg NDMA and/ or 0.022µg AFB1/kg**

| | ALT | | AST | | ALP | | TB | | DB | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PF | PR | PF | PR | PF | PR | PF | PR | PF | PR |
| NDMA | 84.50 ±20.24 | 56.00 ±8.21 | 42.25 ±23.46 | 150.00± 21.73 | 386.00± 92.92 | 281.51 ±19.66 | 51.13 ±16.64 | 32.6 ±10.90 | 33.10 ±3.13 | 23.60 ±1.78 |
| NDMA & AFB1 | 40.00 ±5.83 | 112.75 ±45.40 | 140.00 ±36.04 | 131.00± 41.2 | 292.80± 32.08 | 265.54 ±15.00 | 61.05 ±23.18 | 56.56 ±9.65 | 54.74 ±12.71 | 31.20 ±22.76 |

**Table 5: Activities of some enzymes and biochemical parameters in rats fed low (8%) and high protein diet (64%) following a single oral dose of 16µg NDMA and/ or 0.022µg AFB1/kg**

| | ALT | | AST | | ALP | | TB | | DB | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LP | HP | LP | HP | LP | HP | LP | HP | LP | HP |
| NDMA | 107.00 ±11.94 | 14.40 ±0.60 | 152.51 ±28.72 | 18.16 ±1.12 | 102.12± 11.92 | 66.10 ±0.14 | 44.77 ±5.61 | 54.70 ±0.61 | 19.25 ±2.15 | 25.26 ±0.80 |
| NDMA &AFB1 | 111.5 ±9.57 | 17.87 ±0.42 | 220.0 ±38.30 | 30.90 ±2.20 | 110.40± 14.07 | 70.77 ±0.50 | 67.25 ±9.06 | 14.36 ±0.50 | 44.71 ±7.80 | 6.12 ±1.80 |

## IV. DISCUSSION

The interactions between diet and chemical carcinogens play a part in the geographic and socio-economic variations in tumor incidence in human populations. Biotransformation of foreign chemical compounds including NDMA in the liver has been shown to be influenced by nutritional status. It has been shown that nutritional deficiencies generally cause lowered rates of xenobiotic metabolism [22, 23].

The excretion of a foreign compound in either bile or urine depends especially on the molecular weight of the compound amongst other factors. High molecular weight(>250-300) compounds are known to be excreted less than 5% in urine while low molecular weight compounds are eliminated to about 9-100% in urine. However it should be noted that high excretion of a foreign compounds implies low metabolism rate of such compound

and vice versa. In this study, the results showed that the urinary route is a route of excretion of NDMA. It was observed that protein repletion decreased excretion of NDMA compared to protein free regimen which implies protein free diet reduced rate of NDMA metabolism. This is consistent with previous findings where rats fed on a protein-free high-carbohydrate diet for 7 days metabolized NDMA at only 55% the rate of rats fed on a commercial diet. Dimethylnitrosamine was metabolized by liver slices from rats fed on the protein-free diet at less than half the rate attained by slices from rats fed on a commercial diet [22]. In the presence of AFB1, there was a decrease in the excretion of NDMA compared to when NDMA only was given, in protein free fed rats. This is evident probably because NDMA and AFBI are both metabolized by the cytochrome P450 monooxygenase which suggests competitive mechanism by AFBI. However, an increased excretion of NDMA was found in high protein fed animals compared to their low protein counterparts. Similar reverse effect of AFB1 was found comparing between low and high protein."Table 2 and 3".

It is known that the enzymes: ALT, AST and ALP are mainly found on the liver in high concentration and whenever the enzymes are found in high amounts in the serum, it signifies that the liver has problem . It has been reported that liver cell damage is characterized by a rise in serum enzymes such as AST, ALT, and ALP [24, 25]. This study showed protein repletion decreased the amount of these enzymes in the serum. Similarly, a decrease trend was observed in high protein compared to low protein diet. The considerable leakage of these enzymes into the serum of rats administered with NDMA and AFB1 was an indication of impairments of the excretory function and severe intrahepatic cell damage of the liver "Table 4 and 5". Liver damage is apparent due to the yellow colour that is characteristic or jaundice, and gall bladder becomes swollen. The high level of total and direct bilirubin indicated that the animals were jaundiced [26,27,28].

## V. Conclusion

The level of dietary protein has been shown to affect the metabolism and excretion of nitrosamines. This study has revealed that protein repletion enhanced metabolism of nitrosamines while protein deprivation enhanced their elimination from the body. It has also shown that low protein intake enhanced metabolism of nitrosamines while dietary high protein enhanced their elimination from the body. It has revealed that Aflatoxin B1, a mycotoxin has a reverse effect on nitrosamine metabolism and disposition. Therefore, nutritional status of an individual and the exposure to other chemicals affects the metabolism and excretion of foreign compounds. Hence, staple foods should be screened for the presence of nitrosamines so as to reduce incidences of diseases related to liver damage.

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