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Liver Enzymes and Total Protein Levels as Index of Hepatotoxicity of Naphthalene

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Abstract : *Hepatotoxicity, with subsequent leakage of important liver enzymes has been postulated as one of the mechanisms of naphthalene toxicity. The objective of this study was to investigate the effect of oral administration of naphthalene dissolved in olive oil on metabolism, using male Wistar albino rats, and to compare it to the control group after 14 days of administration. The twelve rats used for the experiment were divided into 4 groups. Groups 1, 2, 3 received 49 mg, 98 mg and 147 mg/kg b.w (body weight) of naphthalene respectively whereas, group 4 (control) took only the vehicle (olive oil). Slight naphthalene hepatotoxicity was evident as the result showed that mean serum ALT, AST and ALP levels were higher in rats administered naphthalene at different concentrations compared to control although, the mean differences were however, not statistically significant (p <.05). Also, mean total protein levels were significantly higher (p<.05) in rats administered naphthalene compared to the control. It can therefore be concluded that the slight elevations in mean serum ALT, AST, ALP activities in groups 1, 2 and 3 are indicative of a possible hepatic dysfunction caused by naphthalene. Elevations in mean total serum protein level are suggestive of chronic inflammation/infection. These results may be pronounced at prolonged administration of naphthalene.*

Keywords: *Hepatotoxicity, Liver Enzymes, Naphthalene*

I. Introduction

Naphthalene (CAS registry number: 91- 20-3) also know as tar camphor, is a slightly water soluble two-ring aromatic hydrocarbon. It exists as white crystalline solid that readily changes from solid to gas at room temperature. It also comprises approximately 10% of coal tar residues at manufactured gas plant (MGP) sites and thus, can be present in soil, and to some degree underground water [1], [2]. Most naphthalene is derived from coal tar and it's the most abundant single component of coal tar. Industrially, distillation of coal tar yields oil containing about 50% naphthalene, along with a variety of other aromatic compounds. Natural occurrences of naphthalene have also been reported. Trace amounts of naphthalene are produced by Magnolias and specific types of deer as well as the Formosan subterranean termite, possibly produced by the termite as a repellant against "ants, poisonous fungi and nematode worms". Naphthalene is also found in light petroleum fractions and in residues from refineries. It is the most volatile member of the polycyclic aromatic hydrocarbons (PAHs), and inhalation is the principal pathway of exposure [3]. Naphthalene has been shown to react readily in the atmosphere with oxidant gases, such as nitrogen oxides and hydroxyl radicals with concentrations of nitro naphthalenes causing air pollution. The metabolism of naphthalene and its respiratory toxicity have been studied extensively and reviewed [4], [5]. The toxicity of naphthalene results from its reactive derivatives through various biochemical steps of which oxidation via cytochrome P450 monooxygenases is the first step, thus producing an unstable 1, 2-epoxide that can convert non-enzymatically (a reaction not involving an enzyme to facilitate) to 1-naphthol (the expoxide) and can also be converted by the enzyme micosomal expoxide hydrolase to naphthalene dihydodiol [6]. Cytochrome P450 enzymes can also facilitate its transformation to naphthalene diepoxides. Likewise glutathione-S-transferase can mediates its transformation to glutathione conjugates. The naphthol and the diol compounds can be further oxidized to form quinines, which, along with the epoxides, represent reactive toxic metabolites that can bind to macromolecules [1]. Naphthalene is used as an intermediate in the production of phthalic anhydride, pharmaceuticals, soil fumigants, insect repellents and other materials [7]. The liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production and detoxification and it reflects the various important biochemical reactions and processes occurring within the body and at the rate they occur. Blood serum or plasma normally contains glucose, proteins, lipids, urea, bilirubin, enzymes etc. However, when different tissues are damaged, the damaged cells tend to release specific enzymes and biochemicals into the blood stream which on measurement with various assaying equipments will result in abnormal levels. For example; the enzyme Alanine amino transferase (ALT) and Aspartate amino transaminase (AST), are enzymes present in liver cells. Damage to the hepatocyte such as in liver disease leads to the released of these enzymes into the blood stream where we can detect and ascertain their abnormal increase which helps

to diagnose and localize the clinical problem involved. Crude oil exploration in Nigeria has brought with it, increased crude oil pollution vis a vis increase in polycyclic aromatic hydrocarbons (PAHs) concentration in atmosphere and our system. Thus, with these high rates of exposure to the PAH comes an increase in the possible risks of human consumption of the PAH prior to its removal. The aim of this investigation was to evaluate the *in vivo* effect of 14 days oral administration of the environmental xenobiotic toxicant, naphthalene, on body chemistry, using selected liver enzymes and total protein as markers in an attempt to ascertain the involvement of naphthalene-induced hepatotoxicity.

II. Material And Methods

1.1 Experimental Animals:

Twelve male Wistar rats were used for the experiment, and were divided into 4 groups. Groups 1, 2, 3 received 49 mg, 98 mg and 147 mg/kg b.w (body weight) of naphthalene respectively, whereas, group 4 (the control) took only the vehicle (olive oil)

2.2 Assay of alanine aminotransferase (ALT) activity

Alanine aminotransferase activity was determined by the Reitman-Frankel colorimetric method for *in vitro* determination [8] in accordance with the method recommended by the International Federation of Clinical Chemistry (IFCC).

2.3 Assay of serum alkaline phosphatase (ALP) activity

Serum alkaline phosphatase activity was determined using Phenolphthalein monophosphate method of [9], [10] in accordance with the method recommended by the International Federation of Clinical Chemistry (IFCC).

2.4 Assay of aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity was determined by the Reitman-Frankel colorimetric method for *in-vitro* determination [8] in accordance with the method recommended by the International Federation of Clinical Chemistry (IFCC).

2.5 Total Protein Determination

A reference Method for measuring total serum protein based on the biuret reaction was used. The method involves a previously described biuret reagent [11] and Standard Reference Material (SRM) 927 bovine albumin (National Bureau of Standards) as the standard [12].

2.6 Statistical Analysis

The results obtained from this study were analyzed using SPSS version 17.0. Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $p<.05$. Posthoc multiple comparisons for differences between groups were established by least significant differences (LSD). All the data are been expressed as mean ± standard error of the mean (SEM) and mean ± standard deviation (SD).

III. Results And Discussion

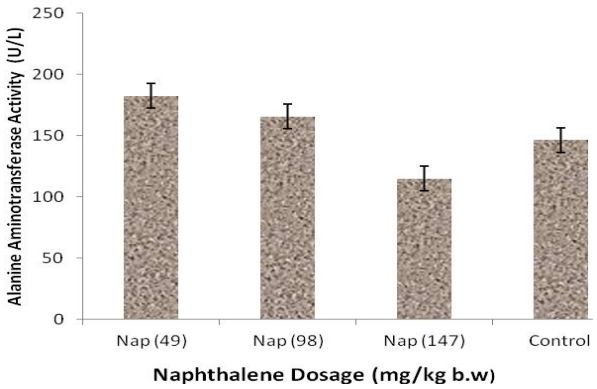

Figure 1: Mean Serum Alanineaminotransferase (ALT) activity (µ/L)

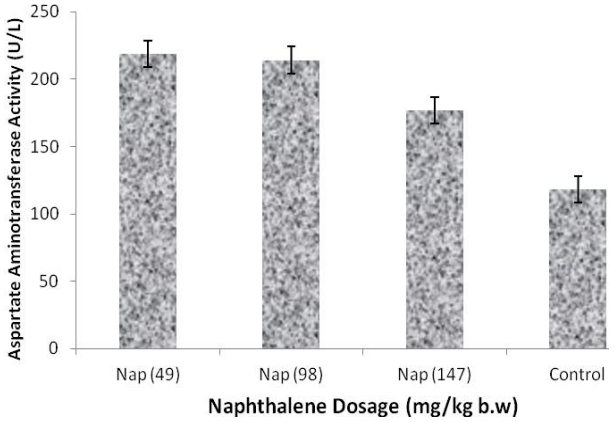

Figure 2.Mean Serum Aspartate aminotransferase (AST) activity (µ/L).

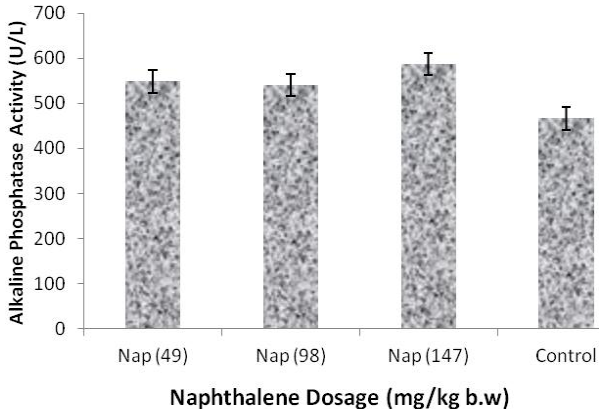

Figure 3: Mean Serum Alkaline Phosphatase (ALP) Activity (µ/L)

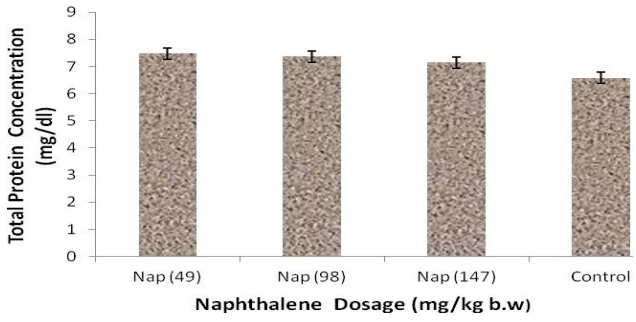

Figure 4: Mean Total Protein Concentration (mg/dl)

Results show that serum activity of ALT is significantly elevated compared to the control. As shown in fig 1, the mean serum ALT levels of the rats fed 49 mg/kg (182.64 ± 38.91) and 98 mg/kg (165.77 ± 74.66) of naphthalene was higher than that of the control (146.57 ± 7.59) although the mean difference was not statistically significant ($p<.05$). According to Reitman and Frankel [8], the elevation of ALT activity appears to reflect some inflammatory disease or hepatic dysfunction (specificity for hepatic disease attributed to biological location of the enzyme), although elevated serum enzyme activity may be seen in extra hepatic diseases. Fig. 2 shows that although the mean difference was not statistically significant ($p<.05$), the mean serum AST activity was moderately higher in rats fed 49 mg/kg (218.70 ± 28.42), 98 mg/kg (214.05 ± 7.86), 147 mg/kg (176.82 ± 31.47) compared to control (118.07 ± 102.43) which might possibly imply manifestation of cellular necrosis

resulting from slight toxicity of the naphthalene. It can also be seen as reported in fig.3 that mean serum ALP activity was higher in rats fed 49 mg/kg (548.63 ± 129.66), 98 mg/kg (540.37± 95.66), 147 mg/kg (587.24 ± 57.56) compared to the control (466.85 ± 96.94). However, the mean difference was not significantly different ($p<.05$) from that of the control rats. It has been reported that ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. Furthermore, ALP is well documented to act as an indicator of cholestatic changes and its activity can serve as indices of biliary damage [13]. Rosalki and Mcintyre [14] also reported that elevations in serum ALP as a reflection of cholestatic disorder, cirrhosis, hepatitis, infiltrative liver diseases etc. In addition, serum total protein levels of rat fed 49 mg/kg (7.47 ± 0.21), 98 mg/kg (7.36 ± 0.23) and 147 mg/kg (7.14 ± 0.23) naphthalene had significantly high ($p<.05$) mean total protein levels compared to the control rats (6.58 ± 0.34) thereby presenting a possible manifestation of chronic inflammation/infection of the liver. Therefore, elevations in mean serum ALT (fig 1), AST (fig 2), ALP (fig 3) activities and total protein (fig. 4) levels at different naphthalene concentration are indicative of hepatic dysfunction, which may be pronounced at prolonged administration.

IV. Conclusion

This report suggest that naphthalene metabolism triggers production of ROS, coupled with impaired oxidant/antioxidant balance, leading to a state of oxidative-stress that could have been partially responsible for the slight hepatotoxicity and the disturbance in the level of hepatic enzymes seen in this study. Therefore, a possible consensus that such biochemical changes observed in these experimental animals may be seen in human beings is undeniable

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