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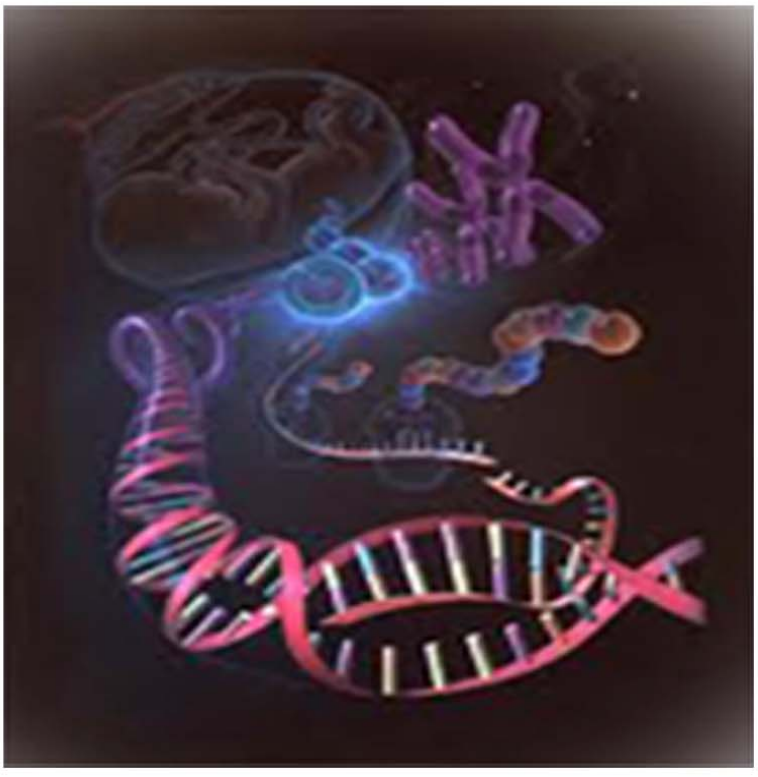

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Research Paper

ALCOHOL INDUCED CHANGES OF RAT SERUM NITRITE AND NITRATE LEVELS

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Nitric Oxide (NO) has been named as molecule of the millennium, Number of authors reported alcohol as to impair the normal functions of cardio-vascular NO pathway in experimental model. We report here the impact of 5 g/kg wt of (20%) alcohol on the rat serum nitrite and nitrate s (NO_2^-/NO_3^-) levels in vivo. Alcohol at the doses administered enhanced rat serum NO_2^-/NO_3^- levels continues that this ascent by way of producing free radical type damage may cause myocardial toxicity in rats.

Keywords: Alcohol, Nitrite, Nitrate, Rat Serum

INTRODUCTION

Forgoing provides in formation regarding the production of NO_2^-/NO_3^- from the NO and its reactions with other molecules in general and under alcoholic stress. In view an attempt was made to determine the serum NO_2^-/NO_3^- levels of both control and alcohol treated rat serum in vivo and the study of these two parameters has been reported to be one of the ways for assessing the overall NO pathway (Guarner, 1993). The data in Table 1 shows that the rat serum receives 5 g/kg of 20% alcohol exihibit an increased levels of both their NO_2^-/NO_3^- levels in a statistically significant ($p<0.001$) manner compared to the normal control group rat serum and the percent increase was found to be more in the rats serum treated with alcohol over 10 weeks period, continuous that the effect of alcohol of rat serum NO_2^-/NO_3^- levels be in a dose and time dependent manner in the control rat serum NO_2^- levels were found to be more than NO_3^- levels and the present observed trend was in accordance with the reports of (Uzun *et al*., 2005; Yugandhar *et al.*, 2011).

MATERIALS AND METHODS

Albino rats of the weight range 125 ± 5 g were selected for the current study. Animals were maintained at a constant temperature of 15 ± 5°C. The rats were divided into three groups of seven each and were maintained in separate cages. They were fed *ad libitum* with commercial rat feed supplied by Kamadhenu Agencies, Bangalore, India.

Treatment of Animals

For *in vitro* studies 20% alcohol 100-1000 µl was

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Table 1: Levels of Nitrite and Nitrate in the Serum of Alcohol Treated Rats (Values Expressed as NG NO_2^- ml of Serum)

Rat Serum	Control	Alcohol Treated	
		5 Weeks	10 Weeks
Nitrite (NO_2^-)Levels			
AV	62.336	71.323	87.287
SD	1.406	1.949	3.373
PC		14.417*	40.028*
t-test			
Nitrate(NO_3^-) Levels			
AV	50.787	68.019	74.270
SD	2.813	2.590	2.806
PC		33.929	46.241
t-test (NO_3^-)			

Note: Values expressed as the mean ± S.D of 6 samples; AV: Average; SD: Standard deviation; PC: Percent change over control; NS: Not Significant; * p< 0.001.

used. For *in vivo* studies group II rats were gavaged with 5 g/kg body of alcohol 20% (w/v) over 5 weeks (weekly doses). Group III rats were gavaged with 5 g/kg body weight of alcohol over 10 weeks (weekly doses) and group I animals acted as normal control ones.

Determination of Serum Nitrite and Nitrite Levels

The serum was collected by centrifuging the blood at 2000 rpm for 10 min. NO_2^- and NO_3^- levels determinants were done according to the procedure of Garner *et al.* (1993). Briefly the control and experimental animal's serum samples were deproteinized before analysis with 35% sulfosalicylic acid. Supernatants of serum were analyzed for NO_2^- concentration, but determination of NO_3^- necessitated reduction of NO_3 to NO_2 which was achieved by reducing the samples in the presence of nitrate reductase and NADPH. The concentration of NO_2 was determined with the Griess reaction. The Griess reagent consisted of one part 0.1% naphthyline diamine dihydrochroride and on part of 0.1% sulfanilamide (sulfanilic acid) in 5% phosphoric acid which were mixed and kept chilled. The color was developed by incubation for 1 h at a 60^0C and the absorbance was measured in a spectrophotometer at 546 nm. Total nitrite and nitrate concentrations were calculated based on the curve obtained with sodium nitrite standards. NO_3^- was determined by substracting NO_2^- values from NO_2^- ± NO_3^- values (measured as NO_2^-). The data was expressed as ng of NO_2^- /ml of serum.

Statistical Analysis

For each parameter, the mean of individual observation (for both control and experimental groups were taken into consideration). Statistical

significance of the data was analyzed through two was ANOVA (analysis of Variance); SNK (Student – Newman - Keuls) test and regression analysis.

RESULTS

The data presented in Table 1 depicts that alcohol at in vivo doses (5 g/kg wt) employed in the present investigation has enhanced both the NO_2^- /NO_3^- levels of rat serum. In the control alcohol treated rat serum NO_2^- levels appeared to be more than the NO_3^- levels (Table 1).

The overall pathway of NO_2^-/NO_3^- formation is as shown below:

$$NO + O_2Hb \rightarrow NO_3^- + Hb^+$$

$$NO + Hb \rightarrow ONOO^- \rightarrow NO_3^-$$

$$2NO + O_2 \rightarrow N_2O_4 \rightarrow NO_2^- + NO_3^-$$

$$NO + Hb \rightarrow NOHb$$

$$No + O_2^- \rightarrow ONOO^- \rightarrow NO_3^-$$

$$2NO + O_{2-}^- \rightarrow N_2O_4 \rightarrow NO_2^- + NO_3^-$$

Since NO_2^-/NO_3^- are being the end products and part and parts of NO pathway, the author attempted to study the in vivo effect of alcohol on rat serum NO_2^-/NO_3^- levels in vivo.

DISCUSSION

Kroncke *et al.* (1997) have reported several cytotoxic effects of NO. NO may react with proteins and nucleic acids. NO has been shown to change ion currents through the mitochondrial membrane leading to release of Ca^{2+} into the cytosol (Nishikawa *et al.,* 1996; Richter *et al;* 1994). In view of the above mentioned cytotoxic effects of NO end products NO_3^- /NO_2^-, excess of NO_2^-/NO_3^- formation induced by alcohol in the present study (Table 1) may cause any of the above stated cytotoxic effects in the myocardial system of rat. Oxidative damage correlates with the amount of ethanol consumed (Clot *et al*., 1994). Recently, it has been demonstrated that NO is an important mediator of hepatotoxicity, and the changes in its generation or actions may contribute to pathologic states (Gardner *et al*., 1998. Diez - Fernandez *et al*., 1998). However, in some models of inflammation including alcoholism, it has been shown that impairment of NO due to alcohol stress increases tissue dysfunction or injury (Kubes *et al.*, 2000) due to formation of NO_2^-/NO_3^- in the serum.

In the present investigation, elevated levels of alcohol treated serum NO_2^-/NO_3^- though difficult to explain the reasons, from the available literature this trend could be due to degradation of organ/ cellular based NO in the rats under alcoholic stress. Identical reports were also made by various authors for agents like cyclosporine (Rajeswara Rao *et al*., 1998), for CCl_4 by Harish Babu (1999); Yugandhar (2011), for organic insecticides by Rajeswara Rao (1997) and for heavy metals by Neelakantam (2008). The excess formation of NO_2^-/NO_3^- in the alcohol treated rat serum may cause free radical type stress in alcohol treated rat tissues in part may lead to myocardial toxicity in rats.

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