



## Decreased Serum Nitrate and Nitrite Levels in Diazepam Treated Rats

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### ABSTRACT

Use of diazepam (DP) has been shown to induce neurotoxic effects. Nitric oxide (NO) has been recently identified as a class of neurotransmitters and has been named as the molecule of the year 1993-94. We measured the NO pathway as its end products nitrite and nitrate ( $\text{NO}_3^- / \text{NO}_2^-$ ) in the serum of rats receiving 2 or 5 mg / kg body wt / animal / over 4 weeks (1 dose / week) by Greiss reaction method. DP decreased the serum  $\text{NO}_3^- / \text{NO}_2^-$  of rats indicating that this agent may lower the production of NO in rats in a dose and time dependent manner.

**Keywords:** Diazepam, Nitrite, Nitrate, Rat Serum, Nitric Oxide.

### INTRODUCTION

Diazepam (DP) is one of the commonly prescribed drugs to treat anxiety (in the elderly populations)<sup>1,2</sup>. This agent is known to exert age-dependent sensitivity in human patients<sup>2</sup>. DP's have specific high affinity stereo specific binding sites in central nervous system (CNS)<sup>4</sup>. Usually a compound like DP that impair the normal functioning of brain should have either direct or indirect impact on one of the newly discovered neurotransmitters like nitric oxide (NO) which is produced stoichiometrically along with citrulline by mediation of the enzyme nitric oxide synthase (NOS) and whose end products were known to be nitrite and nitrate ( $\text{NO}_3^- / \text{NO}_2^-$ )<sup>5-7</sup>. Towards our attempts to investigate the role of various chemical / pharmacologic agents with mammalian NO pathway, we present here the data concerning to the *in vivo* effects of DP on rat serum  $\text{NO}_3^- / \text{NO}_2^-$  levels.

### MATERIALS AND METHODS

#### Chemicals

DP was a product of Ranbaxy laboratories limited, Thane, India. All other chemicals were purchased from sigma chemical company, USA.

#### Treatment of animals

Adult Swiss - albino rats (225 ± 25g) were used in these studies. Administration of food and water were discontinued 24 hours before treatment. Animals (n=6 each) were injected intraperitoneally with DP [2 or 5 mg / kg body weight 4 weeks (weekly doses)] in distilled water, a control group (n6) received the same dose of distilled water. After 4 weeks of treatment, animals were anaesthetized with 50 mg / kg body weight of ketamine hydrochloride and blood was collected through cardiac puncture.

#### Determination of Serum $\text{NO}_2^- / \text{NO}_3^-$

The collection of the serum was done by centrifuging the blood at 2000 rpm / 10 minutes.  $\text{NO}_3^-$  and  $\text{NO}_2^-$  determinations were done using the previous procedure and modified<sup>8</sup>. Briefly, serum samples were deproteinized before analysis with 35% sulfosalicylic acid. Supernatants were assayed directly for  $\text{NO}_2^-$  concentration but determinations of NO required previous reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  which we achieved by reducing the samples in the presence of nitrate reductase and NADPH. The concentration of  $\text{NO}_2^-$  was determined with the Griess reaction. Griess reagent consisted of one part of 0.1% naphthylendiamine dihydrochloride and one part of 0.8% sulphanilamide (or sulfanilic acid) in 5% concentrated phosphoric acid, mixed together and kept chilled. The colour of the end product was developed by incubating the samples at 60°C for one hour. Its absorbance at 546 nm was read in spectrophotometer. Total nitrite and nitrate concentration were calculated based on the standard graph obtained from sodium nitrite. The difference of total and  $\text{NO}_2^-$  were considered as  $\text{NO}_3^-$ .

### RESULTS AND DISCUSSION

The rats receiving 2 or 10 mg / kg body weight of DP showed significantly ( $P < 0.05$ ) decreased serum  $\text{NO}_3^- / \text{NO}_2^-$  levels compared to their control values (Table 1).

Present study has demonstrated that DP administration in rats bring about changes in their NO pathway. NO is an extremely unstable molecule and is readily converted *in vitro* and *in vivo* to  $\text{NO}_3^- / \text{NO}_2^-$ .<sup>9</sup> Psychoactive agents like phencyclidine (PCP), alcohol have been shown to impair the NO pathway in experimental animals. Since DP is identical to alcohol in inducing neurological and other changes he present observed trend of decreased  $\text{NO}_3^- / \text{NO}_2^-$  levels in DP treated rats supports that this agent may lower the production of NO and thus may impair other NO mediated events. How DP behaves in varied pathological states with reference to NO pathway require further studies.<sup>10-13</sup>



**Table 1:** Levels of Nitrite and Nitrate in the Serum of Diazepam treated rats (Values expressed as ng NO<sub>2</sub><sup>-</sup> ml of serum).

Rat Serum	Control	Diazepam Treated (50mg/kg body wt)	
		2 Weeks	5 Weeks
Nitrite (NO <sub>2</sub> <sup>-</sup> ) Levels			
AV		0.64	5.04
SD	37.40	±3.71	±0.97
PC	±5.49	P<0.05	P<0.05
t-test		*	*
Nitrate(NO <sub>3</sub> <sup>-</sup> ) Levels			
AV		6.63	4.56
SD	18.59	±1.81	±1.20
PC	±2.70	P<0.05	P<0.05
t-test		*	*

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

## CONCLUSION

Diazepam administration in rats appear to inhibit the cellular / organ based NO production which may result in lowered serum NO<sub>3</sub><sup>-</sup>/ NO<sub>2</sub><sup>-</sup> pools as observed from our present study.

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