

# In Vitro Action of Tramadol on Biochemical Enzymes in Human Erythrocytes

Ogbomade, R. S.<sup>1</sup>, Asara, A.A.<sup>2</sup>, Eboh, A.S.<sup>3</sup>

<sup>1</sup>*Department of Science Foundation, Bayelsa State College of Health Technology, Otuogidi, Nigeria*

<sup>2,3</sup>*Molecular/Medicinal Phytochemistry Unit, Biochemistry Department, Niger Delta University, Nigeria*

**Abstract:** - In this study the *in vitro* effect of tramadol on the activity of the erythrocyte enzymes AST and ALT, Superoxide Dismutase and Catalase were evaluated. Blood was collected from healthy volunteers and erythrocytes were prepared according to standard procedures and treated with varying concentrations of tramadol (0-0.7mg/ml) and the AST, ALT, CAT and SOD activities were determined by Randox standard kits. Results obtained from the study revealed that administration of tramadol in our study significantly increases the levels of AST and ALT. there was a non-significant difference in the activities of ALT and AST ( $p < 0.05$ ) at 0.1 mg/ml but there was a significant difference at 0.3 mg/ml, 0.5 mg/ml and 0.7 mg/ml as compared with the control. The activity of SOD in the absence of tramadol was  $6.73 \pm 0.01$ . In the presence of tramadol the activity of SOD decreases in a dose dependent manner. The decrease is non-significant ( $p > 0.05$ ) at 0.1 mg/ml and 0.3 mg/ml as compared with the control. This decrease in the activity of SOD could be due to two reasons; Tramadol inhibited the activity of SOD or that at higher concentration tramadol acts as a pro-oxidant producing more free radicals. There are significant decrease of SOD activity at 0.5 and 0.7 ( $p < 0.05$ ) as compared with the control. Tramadol may be recommended to patients with severe pain on prescription but not to be abused because of the side effects associated with an over-dose of the intake of this drug.

## I. INTRODUCTION

The adverse effects associated with drugs used in clinics constitute a serious problem for patients and health care providers (Assis and Nevarro, 2009). It has been estimated that about 10% of drugs are associated with severe, undesirable side effects (Hussaini and Farrington, 2007). However, this number is probably underestimated, given that drug-induced adverse effects are difficult to detect due to pre-existing medical conditions, multiple drug usage, and lack of diagnostic standards (Assis and Nevarro, 2009).

In the last few years, tramadol abuse is steadily increasing in Nigeria and African countries. The prevalence of tramadol in these counties could be attributed to its cheap price, wide availability and illegal smuggling (Fawzi, 2011). The abuse of tramadol overdose has been associated with violence and fights, road accidents and self-induced unintentional injuries (Fawzi, 2011). The side effects of tramadol abuse include confusion, dizziness, seizures, drowsiness and respiratory depression (Clarot et al., 2003). In addition, tramadol abuse can lead to serotonin syndrome (SS), a serious condition

occurs due to excess serotonergic activity and characterized by altered mental status, agitation, tremor, dilated pupils, increased reflexes and other symptoms. long-term use of opioids might induce structural alterations and adverse effects on erythrocytes.

## II. MATERIALS AND METHODS

### *Sample collection and preparation*

Blood was collected by veni-puncture from non-smoker volunteers, who had fasted for at least eight hours. It was then emptied carefully into lithium heparinized sterile bottles.. The preparation of erythrocytes followed the procedure described by Beutler 1984. Briefly, the blood sample was centrifuged at 10, 000 g for 5 minutes, washed thrice with 10 volumes of normal saline (0.9% NaCl) and then diluted 1:20 with a stabilizing solution (0.27 mM EDTA, 0.27 mM 2-mercaptoethanol, pH 7.0). The erythrocyte samples obtained were then frozen and thawed for immediate use, or stored in frozen state until used (usually for not more than 10 days).

### *Effect of Tramadol on RBC in Vitro*

A stock solution of tramadol was prepared (2.5mg/ml) 0mg/ml, 0.1mg/ml, 0.3mg/ml, 0.5mg/ml and 0.7mg/ml of tramadol were prepared from the stock, 100µl RBC and buffer in a total volume of 2ml was incubated at 25<sup>o</sup>C for 1 hr.

### *Enzyme assays*

The assay of AST followed a modification of the colorimetric method reported by Varley *et al* 1980. A 0.1 ml aliquot of haemolyzate was added to 0.5 ml substrate mixture containing 100 mM phosphate buffer (pH 7.4), 2 mM 2-oxoglutarate and 100 mM L Aspartate. Following incubation of the final mixture at 37 <sup>o</sup>C for 30 minutes, the reaction was terminated by the addition of 0.5 ml of 1 mM 2, 4-dinitro phenyl hydrazine in 1 M hydrochloric acid. After allowing the mixture to stand at room temperature for 20 minutes, 5 ml of 0.4 M sodium hydroxide was added. The absorbance of the final mixture was read, after 5 minutes, at 546 nm against a blank solution (no incubation at 37 <sup>o</sup>C, and termination solution added before addition of the haemolysate). Alanine amino transferase assay was carried out as described for AST except that 200mM DL-alanine replaced L-aspartate in the procedure. The activities of AST and ALT were expressed in international units /l.

Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method of Cohen et al. (1970). One milliliter of 50mM phosphate buffer (pH 7.4) and 10  $\mu$ L of hemolysate was added to the cuvette. The reaction was then initiated by the addition of 300  $\mu$ L of 30mM H<sub>2</sub>O<sub>2</sub> prepared by diluting 0.34mL of 30% H<sub>2</sub>O<sub>2</sub> to 100mL of 50mM phosphate buffer (pH 7.4). Specific catalase activities were determined following the changes in the absorbance of H<sub>2</sub>O<sub>2</sub> at 240nm ( $\epsilon = 0.0394 \text{ 1mM}^{-1} \text{ 1cm}^{-1}$  at 240 nm).

Superoxide dismutase assay (SOD) The method used to assay SOD activity is based on the capacity of pyrogallol to autoxidize, a process highly dependent on superoxide (O<sub>2</sub><sup>-</sup>) which is a substrate for SOD (Marllund, 1985 and Kakkar 1984). Briefly, to 15 mL of each sample, 215 mL of a mixture

containing 50 mM Tris buffer, pH 8.2, 1 mM EDTA and 30 mM CAT were added. Subsequently, 20 mL of pyrogallol were added and the absorbance was immediately recorded every 30 s for 3 min at 420 nm using a UV-visible Shimadzu spectrophotometer. The inhibition of autoxidation of pyrogallol occurs in the presence of SOD, whose activity can be indirectly assayed spectrophotometrically. A calibration curve was performed with purified SOD as reference, to calculate the activity of SOD present in the samples. One SOD unit is defined as the amount of SOD necessary to inhibit 50% of pyrogallol autoxidation and the specific activity is reported as SOD units/mg protein.

### III. RESULTS

Table 3.1. Effect of Tramadol on ALT level

Conc. (mg/ml)	ALT (U/l)	AST (U/l)	CAT (U/ml)	SOD (U/ml)
0	31.47±3.36 <sup>a</sup>	36.23±1.96 <sup>a</sup>	43.29±1.69 <sup>a</sup>	6.73±0.01 <sup>a</sup>
0.1	38.67±1.65 <sup>a</sup>	37.98±1.11 <sup>a</sup>	40.80±2.27 <sup>a</sup>	7.12±0.30 <sup>a</sup>
0.3	41.13±1.81 <sup>b</sup>	40.59±0.64 <sup>b</sup>	39.49±1.41 <sup>a</sup>	9.07±0.31 <sup>a</sup>
0.5	48.33±2.04 <sup>b</sup>	50.59±0.70 <sup>b</sup>	37.84±3.89 <sup>a</sup>	10.99±0.21 <sup>b</sup>
0.7	62.80±1.66 <sup>b</sup>	66.87±3.28 <sup>b</sup>	35.29±0.69 <sup>a</sup>	12.27±0.34 <sup>b</sup>

Values are mean of triplicate determination  $\pm$  SD. Values in the same column with different superscripts are significantly different from control (0 mg/ml) ( $p < 0.05$ ).

Result obtained from this study, showed in table 3.1 revealed that there was a non-significant difference in the activity of ALT ( $p < 0.05$ ) between 0 mg/ml to 0.1 mg/ml but there was a significant difference at 0.3 mg/ml and 0.7 mg/ml. There was a non-significant difference in the activity of AST ( $p < 0.05$ ) between 0 mg/ml to 0.3 mg/ml but there was a significant difference at 0.5 mg/ml and 0.7 mg/ml as compared with the control. The activity of Catalase decreases non-significant as the concentration of tramadol increases. The activity of superoxide dismutase increases non-significant as the concentration of tramadol increases.

### IV. DISCUSSION

Elevated activities of AST, ALT are a common sign of liver disease. The levels of AST and ALT are used as diagnostic indicators of hepatic injury. AST and ALT are the most sensitive tests employed in the diagnosis of hepatic diseases. Elevated levels of AST and ALT are indicative of cellular leakage and loss of functional integrity of cell membranes (Kim *et al.*, 2006; Suresh *et al.*, 2006). Administration of tramadol in our study significantly increases the levels of AST and ALT. there was a non-significant difference in the activity of ALT ( $p < 0.05$ ) between 0 mg/ml to 0.1 mg/ml but there was a significant difference at 0.3 mg/ml and 0.7 mg/ml. There was a non-significant difference in the activity of AST ( $p > 0.05$ ) between 0 mg/ml to 0.3 mg/ml but there was a

significant difference at 0.5 mg/ml and 0.7 mg/ml as compared with the control.

Superoxide dismutase (SOD) is the first detoxification enzyme and most powerful antioxidant in the cell. It is an important endogenous antioxidant enzyme that acts as a component of first line defense system against reactive oxygen species (ROS). It catalyzes the dismutation of two molecules of superoxide anion (O<sub>2</sub><sup>-</sup>) to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and molecular oxygen (O<sub>2</sub>), consequently rendering the potentially harmful superoxide anion less hazardous (Fridovich, 1995). The activity of SOD in the absence of tramadol was 6.73±0.01. In the presence of tramadol the activity of SOD decreases in a dose dependent manner. The decrease is non-significant ( $p > 0.05$ ) at 0.1 mg/ml and 0.3 mg/ml as compared with the control. This decrease in the activity of SOD could be due to two reasons; Tramadol inhibited the activity of SOD or that at higher concentration tramadol acts as a pro-oxidant producing more free radicals. There are significant decrease of SOD activity at 0.5 and 0.7 ( $p < 0.05$ ) as compared with the control.

Catalase CAT is a common antioxidant enzyme present almost in all living tissues that utilize oxygen. The enzyme uses either iron or manganese as a cofactor and catalyzes the degradation or reduction of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to water and molecular oxygen, consequently completing the detoxification process imitated by SOD (Marklund, 1984). The activity of catalase decreases non-significantly ( $p > 0.05$ )

as the concentration of tramadol increases. This may be that as the concentration of tramadol increases it inhibited catalase.

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