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BIOCHEMICAL INVESTIGATIONSOF THE EFFECT OF XANAX ON THE CEREBELLAR TISSUES OF MALE MICE

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Abstract:-

Xanax is an agent with hypnogenic, anxiolytic, anticonvulsant, and muscle relaxant properties and has generally been used as a hypnotic/tranquilizer. The aim of this paper was to investigate the effect of Xanax on acetylcholinesterase and glutathionestransferase enzyme activities on the cerebellar tissues of male mice. Sixty male mice were randomly assigned into four groups (15 mice/each) according to their approximately equal mean body weight. Mice that received orally by gavage 0.5 ml saline solution of 0.9% NaCl were considered as a control mice. Other experimental mice were daily administered orally by gavage with 0.5 ml of different three doses of Xanax (0.5, 1 and 1.5 mg/kg bw), for two months. Biochemical analyses revealed significant decreases in the activities of acetylcholinesterase and glutathionestransferase enzyme in the brain tissues of mice administered with the three doses of Xanax. The major mechanism involved appears to be the result of oxidative stress scavenging action on the neuronal cells of cerebellar cortex of male mice.

Key words: - *Xanax, Acetylcholinesterase, Glutathione Stransferase, Cerebellar, Mice.*

INTRODUCTION

The medical literature mentions very few data about the analgesic or algesic effects of benzodiazepines or other drugs with similar mechanism of action.Anxiety is an aversive emotional state with the apprehensive anticipation of indefinite threat or danger [1]. It particularly arises when an individual is involved in a situation which is subjectively relevant and anticipated as threatening, ambiguous, uncontrollable, unpredictable and novel [2]. Xanax is the most widely prescribed benzodiazepine (BZD) and is used as an anxiolytic, antipanic, and antidepressant agent [3-4]. In humans, Xanax is rapidly and completely absorbed after oral administration, with an elimination half-life of 6 to 16 h and volume of distribution of l/kg, respectively [4-6-7]. It acts by binding to benzodiazepinebinding sites on $GABA_A$-receptors and stimulates the inhibitory action of gamma-aminobutyric acid (GABA) in central nervous system (CNS) [8–9]. The mechanisms of BZD action at molecular level involve binding of BZD to specific BZDbinding site at gamma butyric acid receptor A ($GABA_A$), which works as ligand-gated channel for Cl^- ions [10-11]. Binding of BZD to $GABA_A$ promotes the effects of GABA on $GABA_A$ receptor and consequently increases the conductance of Cl^- across the neuronal cell membrane, increases membrane potential and inhibits neuronal firing [12]. Acetylcholine is a major neurotransmitter found in many organisms including humans. Acetylcholinesterase (AchE) hydrolyzes the neurotransmitter acetylcholine, and it is found at mainly neuromuscular junctions and cholinergic brain synapses, where its activity serves to terminate synaptic transmission. The activity of AChE is higher in motor neurons than in sensory neurons. This enzyme activates muscles in the peripheral nervous system [13], and it has other effects on neurons, where it might cause a slow depolarization by blocking a tonically-active K^+ current, which increases neuronal excitability. Although acetylcholine induces contraction of skeletal muscle, it acts via a different type of receptor (muscarinic) to inhibit contraction of cardiac muscle fibres [14]. Oxidative stress is discussed as a contributor to the initiation or progression of cellular damage and has been implicated in the pathophsiology of many neurodegenerative diseases by inducing the reactive oxygen species (ROS) that oxidize vital cellular components such as lipids, proteins and DNA which produces potentially harmful effects [15]. The cerebellum plays an important role in motor control, and it is involved in some cognitive functions such as attention and language, and probably in some emotional functions such as regulating fear and pleasure responses [16]. So, the aim of this study was to evaluate the biochemical findings on the cerebellar tissues of male mice.

MATERIALS AND METHODS

Drugs:

Xanax (Kalma) was purchased from the local pharmacy, and produced by Amoun Pharmaceutical Company, Egypt, in the form of tablets. Each tablet contains 0.5 mg Xanax, and it was dissolved in saline solution (0.9 % NaCl).

Study design:

Thirty male mice albino male mice, weighing approximately 30 ± 3 g each. Animals were maintained at the animal care facility in stainless steel cages (5 mice/cage). All mice were adapted to the controlled environmental conditions at room temperature of 25 ± 2 °C, relative humidity 60-70 %, and normal photoperiod 12 h/d. Also, they were allowed free access of food and drinking water *ad libitum*.Mice were acclimatized to the laboratory environment for two weeks prior to the starting of the experiment. Sixty male mice were randomly assigned into four groups (15 mice/each) according to their approximately equal mean body weight. Mice that received orally by gavage 0.5 ml saline solution of 0.9% NaCl were considered as a control mice. Other experimental mice were daily administered orally by gavage with 0.5 ml of different three doses of Xanax (0.5, 1 and 1.5 mg/kg bw), for two months [17].

Twenty-four hours after last treatment, the animals were euthanized using diethyl ether, the brains of the control and experimental treated mice were immediately removed and washed in ice-cold glass slides. The brain tissues were homogenized separately in 10 volumes (w/v) of 0.1M phosphate buffer pH 7.4 using a polytron homogenizer for one minute. The homogenates were centrifuged at 4000 r.p.m. for 20 minutes using CRU-5000 centrifuge, and refrigerated at 4°C [18]. The homogenate and supernatant were used for estimation of the activities of both acetylcholine enzyme (AChE) and the total glutathione trasferase (GST).

Biochemical analysis:

Acetylcholinesterase activity:

Acetylcholinesterase activity was measured by the method of [19]. This method is based on the hydrolysis of acetylthiocholine iodide (ATChI) as substrate by theenzyme to produce thiocholine and acetic acid. Thiocholine reacts with dithiobisnitrobenzoate (DTNB) to produce the yellow anion of 5-thio-2-nitrobenzoic acid.

Glutathion S transferase enzyme activity:

Glutathion S transferase enzyme activity was assayed by the method of [20].The biodiagnostic glutathione S- transferase assay kit measures the total GST activity by measuring the conjugation of 1-chloro- 2, 4- dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST enzyme activity in the sample.

Statistical analysis:

Statistical analysis was performed using the statistical package for social science (SPSS) version 18.0 statistical analysis package. Parameters were analyzed using significance by one way analysis of variance (ANOVA) were performed and variant groups were determined by means of the Duncan test. *P* value was assumed to be significant at 0.05.

RESULTS

Acetylcholine esterase enzyme (AChE) activity:

The results shown significant decrease in the level of acetylcholine enzyme activity in the brain tissue of mice administered with the three different doses of Xanax, comparing to the control this decrease was a dose dependent, and the mean value of the level of AChE in the brain tissue of control mice was 280.0 ± 22.65, decreased gradually to 230.5 ± 21.72, 187.5 ± 19.65 and 108.0 ± 11.07 in the brain tissues of mice treated with 0.5, 1 and 1.5 mg/ kg bw Xanax for two months **(Table 1 and Fig. 1).**

Total glutathione transferase (GST) activity:

Significant decreases in the activities of GST, were noted in the brain tissues of mice administered with the three doses of Xanax. This decrease was a dose dependent. The mean value of GST in the brain tissues of control mice was 16.25 ± 1.25, decreased gradually to 11.6 ± 1.33, 10.15 ± 1.07 and 8.7 ± 0.97 in mice treated with 0.5, 1 and 1.5 mg/kg Xanax for two months**(Table 1 and Fig.**

Table (1): Activity of acetylcholine esterase and total glutathione in the cerebellar tissues of mice treated with different doses of Xanax and the control.

Experimental Groups	ACHE	GST
Control	280.0 ± 22.65^{a}	16.25 ± 1.25^{a}
0.5 mg/kg bwXanax	230.5 ± 21.72	11.6 ± 1.33^{b}
1 mg/kg bwXanax	187.5 ± 19.65^{b}	10.15 ± 1.07^{b}
1.5 mg/kg bwXanax	108.0 ± 11.07^{c}	8.7 ± 0.965^{b}

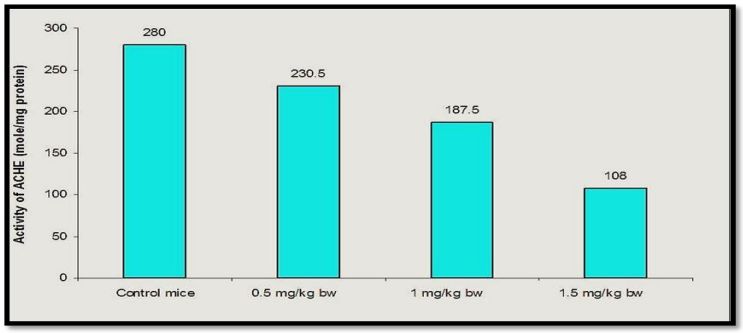

Fig (1): Activity of acetylcholine esterase in the cerebellar tissues of mice treated with different doses of Xanax and the control.

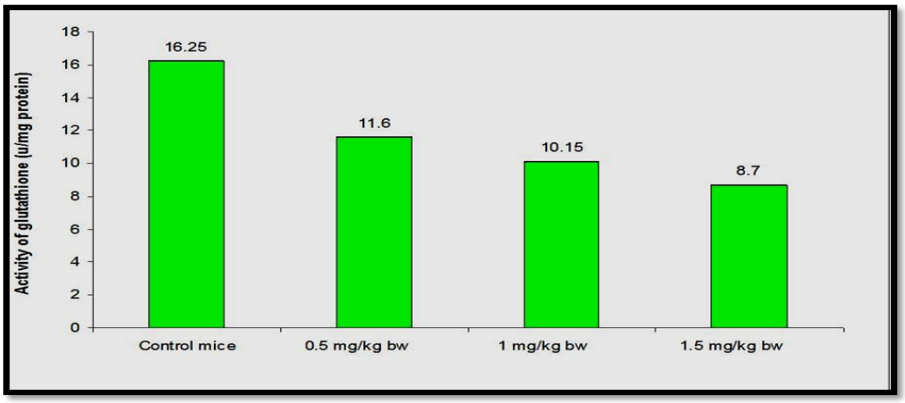

Fig (2): Total glutathione activity in cerebellar tissues of mice treated with different doses of Xanax and the control.

DISCUSSION

The oxygen radicals that are normally produced within the body are usually kept in check by complex multifactorial protective enzymes, which including the superoxide dismustase, catalases, and glutathione peroxidase, which can check the free radicals originating either in the mitochondria or in the cytoplasm. The brain, compared to liver, lung and other organs, contains relatively low levels of enzymatic and non-enzymatic antioxidants and high amounts of peroxidizable polyunsaturated lipids, rendering it more vulnerable to oxidative stress compared to other tissues [21-15]. Moreover, the brain exhibits distinct variations in cellular as well as regional distribution of antioxidant biochemical defenses [22]. Thus, neural cells and/or brain regions are likely to differentially respond to changes in metabolic rates associated with the

generation of ROS [23]. There is abundant evidence invoking regional sensitivity to oxidative stress that is dependent on cellular and regional redox status [24].
In our study we detected significantly decreases in the activity of the total glutathione, which was in proportional with the decrease in the activity of acetylcholine esterase in the brain tissues of the treated mice. The cellular glutathione is the most abundant low molecular weight thiol involved in antioxidant defense in animal cells, and it is the major antioxidant compound that acts directly both in removing ROS and as a substrate for several peroxidases. Therefore, it can be said that increase in ROS production due to exposure to Xanax led to the decrease in the level of acetylcholine esterase activity. This is confirmed with the suggestion of [25] who reported that the activity of acetylcholine esterase was inhibited by free radical formation. Feoli *et al.,* [26] suggested that glutathione deficiency contributes to oxidative stress in many brain disorders, including seizure and stroke, as well as in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Stress has been shown to affect several brain activities and promote long-term changes in multiple neural systems [27]. Levi and Basuaj [28] found that stress has been shown to cause a decrease in the level of glutathione which protect the tissues from oxidative damage. Gilgun-Sherki *et al.,* [29] suggested that ROS attack glial cellsand neurons which are post-mitotic cells, and therefore they are particularly sensitive to free radicals, leading to neuronal damage.

So, the current results are in agreement with the suggestion of [30-31] who attributed the cause of oxidative stress in the brain tissue is thought to be collectively after sodium fluoride treatment could cause oxidative stress, apoptosis, and decreased mRNA and protein expression levels of neural cell adhesion molecules in rat neurons, contributing to the neuronal dysfunction and synaptic injury.

Along with glutathione peroxidase (GSH-Px), GST activity was found to be lowered in brain regions of PPr-exposed animals. Free radicals induce tissue damage by initiating and propagating lipid peroxidation (LPO) [32]. The increase in LPO in brain regions observed in present experiment could be due to the significant increase in the generation of ROS in those regions of PPr-exposed rats. It may be one of the reasons for decreased weight of cerebellum, cortex and hippocampus in PPr-exposed animals. According to the results of the present study, it can be concluded that Xanax induced marked decrease in acetylcholine esterase activity in the brain tissue. Also, the current study demonstrates that the enzymatic antioxidant GST activity was significantly decreased by Xanax. Further studies need to address the exact mechanisms of the dissociation between the different response systems in healthy subjects.

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