International Journal of Medical and Health Research
www.medicalsciencejournal.com
ISSN: 2454-9142
Received: 20-08-2022, Accepted: 05-09-2022, Published: 23-09-2022
Volume 8, Issue 5, 2022, Page No. 16-18

Effect of diazepam on oxidative stress and some antioxidant markers with liver enzymes in male rabbits

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Abstract
This study aimed at investigating the impact of Diazepam on serum liver enzyme, oxidative stressm, and som antioxidant markers in male rabbits.
Methods: A total of 20 growing rabbits (7.5 weeks old) reared under high ambient temperature were divided into two equal groups, 10 rabbits each. The first group control administered with normal saline and the second group Diazepam-treated rats (50 mg/kg b. w. orally) for 30. Blood samples were withdrawn to measure serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Glutathione (GSH), malondialdehyde (MDA) levels, and catalase (CAT), activities were assayed.
Results: In Diazepam -treated group, significant increases in aspartate aminotransferases, and alanin aminotransferases activities, malondialdehyde (MDA) levels and significant decreases in catalase (CAT), glutathione activities levels were determined compared to the control group.
Conclusion: Diazepam administration produces noticeable biochemical changes in a dosedependent manner associated with increased liver enzyme and oxidative stress markers and decreased antioxidative activity.

Keywords: diazepam, malondialdehyde, hepatic toxicity, GSH

Introduction

Anxiolytics are prescribed worldwide for the relief of pain, of which benzodiazepines are among the most frequently prescribed [1]. diazepines (BDZ) Diazepam (7-chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one) are the most frequently prescribed class of psychotropic drugs may be worldwide [2]. Commonly used for several neurological disorders like seizures, anxiety, er, restless legs syndrome and spasm of muscle. It is also used for purposely loss of memory during various medical surgeries [3]. The central diazepine receptor is mainly present in the central nervous system (CNS) and forms part of the γ-aminobutyric acid $(GABA)_A$ receptor complex. The peripheral benzodiazepine receptor is a ubiquitously expressed protein of the outer mitochondrial membrane termed translocator protein 18 kDa (TSPO), structurally and functionally different from the $GABA_A$ receptor [4]. Onset of action is fast after intravenous injection but slow to intermediate after oral or rectal administration. The half-life is longer than 24 hours, although anticonvulsant effects and sedation are often shorter as a result of redistribution from the CNS. It is metabolized by liver cytochrome P450s to three major active metabolites; N-desmethyldiazepam or nordiazepam (NDZ), oxazepam (OX) and temazepam (TZ), which are conjugated and excreted mainly as glucuoronide in urine [5]. Oxidative stress, a pathophysiologic imbalance in the production of oxidants or free radicals and antioxidant molecules in the body would result in OS state [6]. Reactive oxygen species (ROS) have many physiological regulatory functions and are also implicated in the development of a wide spectrum of diseases [7].
In the present study was carried out to investigate the biochemical changes in the liver enzyme and oxidative stress, and antioxidant enzymes of rats exposed to high doses of paracetamol.

Material and Methods

Twenty healthy male rabbits weighing about (1.5–2.3 kg) g with an average age of 75±5 days. purchased from the center for experimental animals, Faculty of Veterinarian Medicine, Tikrit University were used in the study. This study was performed in accordance with the Guide for the ethical care and use of laboratory animals.
They were left to acclimatize for 1 week. They were housed at room temperature in metallic cages and were kept under constant healthy environmental and nutritional conditions. Animals were kept under a schedule of diurnal lighting conditions (12 h of darkness and 12 h of light); they were fed on ordinary food and housed under standard laboratory conditions.

The animals were divided into 10 cages of 5 animals each.
Group I (positive control group) with saline: Each rabbit received 1 ml of normal saline/day, orally for 30 days.
Group II (Diazepam-treated group): 10 rabbits were treated with Diazepam (50 mg/kg/day), orally for 30 days.
Blood samples (5 ml) were collected through the retro-orbital puncture, serum samples were prepared for biochemical assays by centrifuging for 10 min at 3000 rpm. All serum samples were kept at −80°C until the assays were performed.
Aspartate aminotransferase (AST), ALP, and alanine aminotransferase (ALT) were measured by using kits from a product of Randox Laboratories, UK). Serum SOD, GSH, MDA, G-Px, and catalase levels were measured by spectrophotometric kit.

Statistical Evaluation

Data were expressed as mean ± SD. Differences between groups were compared by ANOVA using the SPSS software (version 16). A p-value of less than 0.05 was considered to be statistically significant.

Result

Table 1: Effect of Diazepam administration on AST, ALT, and ALP of rabbits

Parameters	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
control	86.60 ± 2.91	71.70 ± 2.39	137.39±0.80
Diazepam	184.50 ± 8.09[a]	217.5 ± 11.47[a]	186.19± 1.70[a]

Table 1 shows highly significant increase in the mean values of serum AST, ALT, and ALP level in the Diazepam treated groups when compared to the control group (184.50 ± 8.09vs 86.60 ± 2.91 IU/L: P < 0.01), (217.5 ± 11.47 vs 71.70 ± 2.39 IU/L: P < 0.01), and (186.19± 1.70vs 137.39±0.80: P < 0.01).

Table 2: Effect of Diazepam administration on catalase and glutathione and MDA of rabbits

Parameters	Catalase (K/ml)	GSH (mg/mg protein)	MDA (mol/L)
Control	52.40 ± 0.86	59 ± 0.04	4.74 ± 0.35
Diazepam	40.50 ± 1.01[b]	0.26 ± 0.03	9.45 ± 0.33 [a]

Treatment of rabbits with Diazepam resulted in a decrease (p < 0.05) in catalase and glutathione activities when compared to the control group (40.50 ± 1.01
vs 52.09 ± 0.84 K/ml: P < 0.01), and (0.26 ± 0.03 vs 0.63 ± 0.05 mg/mg protein: P < 0.01). Whereas it markedly increased MDA levels in the serum of treated rabbits compared to control rabbits (9.45 ± 0.33 vs 4.74 ± 0.35 mol/L: P < 0.01). as shown in Table2.

Discussion

Aspartate aminotransferases are predominantly mitochondrial enzymes. Although an elevated level of AST in the serum is not specific for a hepatic disorder, it is used primarily to diagnose and confirm persistent cellular injury in conjunction with other enzymes such as ALT [8, 9]. is the most reliable biochemical value to show the of liver injury in patients with acute and chronic viral hepatitis, this is due to the distribution of cytoplasmic exclusively of ALT and longer half-life in the blood (about 50 hours) than for AST (about 16 hours) [10].

In the current study an increase in the activities of AST, and ALT in serum might be mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells. For it, the marked release of AST and ALT from liver cytosol into circulation refers to the extensive damage of hepatic tissue membranes during toxicity [11, 12].

The specific role of antioxidants is to neutralize rampaging free radicals and thus reduce their capacity to damage. They act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, and synergists, which can provide an indication of the level of stress experienced in a cell or tissue [13, 14]. Glutathione has a role in scavenging ROS and reactive nitrogen species. There was a marked reduction in GSH level in the diazepine treated groups compared to the control. Depletion of GSH in tissue indicates its utilization and thus increased tissue susceptibility to oxidative stress [15, 16].

Malondialdehyde (MDA): dienconjugation (DC) and thiobarbituic acid reactive species (TBARS) are widely as indicators of lipid peroxidation, DC is a measure of early events of lipid peroxidation reaction where TBARS measure end product of lipid peroxidation (MDA) that is a goal market of cell membrane damage following ROS production during stress [17]. Malondialdehyde is a decomposition product of auto oxidation of polyunsaturated fatty acids which is used as an index of oxidative damage. The high concentration of MDA in those patients indicates increased membrane lipid peroxidation.As regard serum MDA level which decomposition product of auto oxidation of polyunsaturated fatty acids iused asindex of lipid peroxidation and oxidative stress was highly significantly increased in Diazepam dependent (group II) as compared to the control (group I).

The tisuee is particularly vulnerable to oxidative stress because it consumes large amounts of oxygen, has abundant lipid content, yet maintains a relative paucity of antioxidant levels [18-20].

Conclusion

Diazepam administration produces noticeable biochemical changes in a dose-dependent manner associated with increased liver enzyme and oxidative stress markers and decreased antioxidative activity.

References

1. Nzor Joyce *et al*. "Impact of benzodiazepines administration on selected biochemical parameters of albino Wistar rats (Rattus rattus)." Egyptian Pharmaceutical Journal,2018:17(1):40. Gale Academic OneFile, link.gale.com/apps/doc/A537808753/AONE?u=googlescholar&sid=googleScholar&xid=310ee250. Accessed 20 July 2022.
2. El-Sokkary GH, Hareedy HHG, Youns HAM. Protective role of melatonin on the effect of diazepam on proliferative activity, morphological changes and testosterone levels in the testes of rats. J Histol Histopathol,2018:5:5. http://dx.doi.org/10.7243/2055-091X-5-5
3. Al-Abbasi FA, Kumar V, Anwar F. Biochemical and toxicological effect of diazepam in stress-induced cardiac dysfunctions. Toxicol Rep.,2020:7:788-794. doi: 10.1016/j.toxrep.2020.06.004. PMID: 32642445; PMCID: PMC7334438.
4. Falcón CR, Hurst NF, Vivinetto AL, López PHH, Zurita A, Gatti G, *et al*. Diazepam Impairs Innate and Adaptive Immune Responses and Ameliorates Experimental Autoimmune Encephalomyelitis, 2021.
5. Mohammad-Reza Rouini, Yalda H Ardakani, Kambiz A Moghaddam, Solatani F. An improved HPLC method for rapid quantitation of diazepam and its major metabolites in human plasma. Talanta, 75(2008), 671-676.
6. Mahde S Hamad, Entedhar R Sarhat, Zubaidah NM Albarzanji. Saliva as a Biomarker in Oral Cancer. Tikrit Journal for Dental Sciences,2022:10(1):75-79.
7. Sarhat ER, Siham A Wadi, Mutaz S Ahmed, Shaima N Mustafa, Thuraia R Sarhat. Evaluation of Serum

Malondialdehyde, Glutathione peroxidase, Superoxide dismutase, and Catalase levels in Hormonal Contraceptives. Tikrit Medical Journal,2018:24(1):10-20.
8. Sayran Sattar Saleh, Entedhar Rifaat Sarhat. Effects of Ethanolic Moringa Oleifera Extract on Melatonin, Liver and Kidney Function Tests in Alloxan-Induced Diabetic Rats. Indian Journal of Forensic Medicine & Toxicology,2019:13(4):1015-1019.
9. Sarhat ER, Zbaar SA, Ahmed SE, Ahmed TS, Sarhat TR. Salivary biochemical variables of Liver Function in among Individuals with COVID-19 in Thi-Qar Province. Egyptian Journal of Chemistry,2022:65(6):305-310.
10. Nawal A Al- Madany, Entedhar R Sarhat. Determination of Some Biochemical Parameters of Patients with Hepatitis B in Kirkuk City.KUJSS,2018:13(2):139-148.
11. Entedhar R Sarhat, Siham A Wadi, Saba K Ibrahim. The Influence of Lycopene on Interleukin-6, Tumor Necrosis Factor -α, Alanine Aminotransferase, Aspartate Aminotransferase Levels in Stereptozotocin - Induced Diabetic Rabbits. 3 rd Scientific Conference - College of Veterinary Medicine - University of Tikrit,2016:2:1-5.
12. Sarhat ER, Wadi SA, Sedeeq BI, Sarhat THR, NA. Study of histopathological and biochemical effect of Punica granatum L. extract on streptozotocin -induced diabetes in rabbits. Iraqi Journal of Veterinary Sciences,2019:33(2):189-194. doi: 10.33899/ijvs.2019.125523.1045
13. Neda Awni. Takea Shaker Ahmed, Entedhar R Sarhat, Nagham Hasan Ali, Kasim Sakran Abass. Altered serum levels of melatonin, antioxidant enzymes and oxidative stress in individuals with diabetes mellitus type 2. Revista Latinoamericana de Hipertensión,2022:17:138-141.
14. Entedhar Riffat Sarhat. Effect of Ginger on the activity of some antioxidant enzymes (Superoxide dismutase, and Catalase) of Alloxan Experimental Induced-Diabetic Rabbits. Al-Mustansiriyah J. Sci.,2011:22(5):192-200.
15. Entedhar R Sarhata, Moayad M Al Anzy, Takea Shaker Ahmed. Study of oxidant-antioxidant status in cerebrospinal fluid of children with meningitis. Eurasian Chemical Communications,2022:4(9):863-869. Link: http://www.echemcom.com/article_148799.html
16. Intesar J. Mohammed, Entedhar R. Sarhat, Siham A. Wadee, Salwa M Al-Shiakhani. Histological and Biochemical Evaluation of the Effect of Desloratadine Drug in Parotid Gland Tissues. Al- Anbar Medical Journal,2021:17(2):72-77. 10.33091/amj.2022.171074
17. Entedhar R Sarhat, Buthyna A Abdullah2, Siham A Wadi. Experimental Study of Effect of Ginger Ethanolic extract on Oxidative Stress in Alloxan Induced-Diabetic Rabbits. 3 rd Scientific Conference - College of Veterinary Medicine - University of Tikrit,2016:2:35-38.
18. Öznur Güngör, Nurhayat Özdemir. Analysis of Oxidative Stress Parameters Depend on Diazepam Application in Rat Serum and Tissue Samples. Chem Sci Trans.,2014:3(1):389-395. DOI:10.7598/cst2014.616
19. Khalid G Washeel, Entedhar R Sarhat, Talal H Jabir. Assessment of melatonin and oxidant-antioxidant markers in infertile men in Thi-Qar Province. Indian Journal of Forensic Medicine & Toxicology,2019:13(4):1495-1499.
20. Sarhat ER, Intesar J Mohammed, Noor Y Mohammed, Bdoor S Khairy, Ghosoon F Hassan. Evaluation of Salivary Oxidative Stress Marker (Lipid Peroxidation), and Non-Enzymatic Antioxidants (Vitamin C and Vitamin E) in Patients with Acute Myocardial Infarction. Tikrit Journal for Dental Sciences,2019:7(1):20-26.