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Original Research Article

Effect of Diazepam on Salivary Gland (Parotid Gland) in Rats

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Abstract: ***Background*:** Diazepines, the most commonly prescribed psychotropic drugs with anxiolytic action, may cause hyposalivation. ***Aim of Study*:** Is to throw light on the structural alternation in the rat parotid gland after administration of diazepam. ***Methods*:** Thirty male Wistar rats were divided into three groups. Control groups received a saline solution for 30 days. Group two and Group three salivary tissue was taken for histological assessment and biochemical assays. ***Results*:** The serous acinar cells were showed autolysis and nuclear changes (pyknosis, karyorrhexis, and karyolysis). There was an increase in the interstitial spaces between each parenchymal element associated with few mononuclear cell infiltrations. The intra-lobular ducts were reduced in size and were indistinct throughout lobes. Malondialdyhid (MDA) concentration had been increaseh high Significant ($P \leq 0.01$) in diazepam group of animals compared with control group. The diazepam group showed significantly decrease levels of GPX, SOD, GSH and CAT compared with the control group. ***Conclusion*:** Diazepam administration produces noticeable histological changes in a dosedependent manner associated with increased oxidative stress markers.

Keywords: Diazepam, Histology, Parotid gland, oxidative stress.

INTRODUCTION

The parotid and submandibular glands are compound tubuloacinar exocrine glands that secrete a high proportion of the saliva into the oral cavity. Mammalian salivary glands are exocrine glands that produce saliva through a system of ducts. There are three pairs of major salivary glands: the sublingual glands, the submandibular glands, and the parotid glands, are compound tubuloacinar exocrine glands that secrete a high proportion of the saliva into the oral cavity and are located in front of the ears and extend to the area beneath the earlobes along the lower border of the jaw [1-3].

Diazepam, which was initially sold under the brand name Valium, is a belongs to a class of drugs called benzodiazepines, is a positive allosteric modulator of the γ-amino butyric acid (GABA), receptors, It is indirectly enhances binding affinity of gamma-aminobutyric acid (GABA) to ligand-gated GABAA receptors to exert its pharmacological effects. Benzodiazepines act as depressants on the central nervous system since they facilitate binding of GABA to various GABA receptors throughout the central nervous system [4-7]. GABA is a neurotransmitter inhibitor which will block impulse transmission in nerve fibers. GABA will open chloride ion gate which is negatively charged thus nerve fibers will be highly negatively charged.

Diazepines are responsible for increasing the frequency of the open state of this receptor channel. Increased GABA receptor activity, Therefore, it's difficult to transmit impulses through nerve fibers when the neuron inhibition of GABA stronger. Continuous use of diazepam can cause side effects, especially in brain, livers and kidneys [8-11]. Diazepines commonly used for a number of neurological disorders like seizures, anxiety, benzodiazepine withdrawal syndrome, alcohol withdrawal syndrome, sleeping disorder, restless legs syndrome and spasm of muscle [12, 13]. Benzodiazapenes have a direct inhibitory effect on the proliferation and production of anti-inflammatory cytokines and activation of T lymphocytes isolated from lymph nodes.

Diazepam reduced the stress response and anxiety related conditions through its effect upon the hypothalamus and/or pituitary gland directly without the involvement of internal tissue. Additionally, this drug influences the activity of the hypothalamus-pituitary-adrenal axis in humans by reducing basal ACTH and cortisol release [9].

Diazepam is a long-acting benzodiazepine metabolized in both humans and rodents by is N-demethylated by CYP3A4 and 2C19 to the active metabolite N-desmethyldiazepam, and is hydroxylated by CYP3A4 to the active metabolite temazepam. N-desmethyldiazepam and temazepam are both further metabolized to oxazepam. Temazepam and oxazepam are largely eliminated by glucuronidation [10-13].

Diazepam considerable information has been reported on the adverse effects of benzodiazepines on the parotid gland, with limited information of its effect on biochemical parameters. The salivary glands are largely regulated by the autonomic nervous system (ANS); parasympathetic and sympathetic nerves control the secretion of saliva by acting through receptors found on salivary acinar cells. In addition to the control of the glands by the ANS, some gastrointestinal hormones can also affect the composition and flow rate of saliva [14-16]. Drug-induced salivary complications are very common, especially in polymedicated and long-course treated elderly patients [17].

Diazepam act to induce reduction in salivary flow through GABAA receptors located in the salivary glands as well as through indirect actions of the CNS on the salivary glands mediating central GABAA receptors. In addition to inhibiting the muscarinic receptors present in the salivary glands, which are responsible for the salivary flow, BZDs also can affect the transport of chloride and the calcium influx, which can lead to fluid secretion. As a result, a reduction in the salivary secretion activity occurs. BZDs can also inhibit β-adrenergic receptors, in turn blocking the release of amylase from rat parotid glands [18].

METHODS AND MATERIALS

Animals

Therty albino Wistar male rats approximate weight 140−160 g was procured from the Animal House facility of Department of Biochemistry, Vetrinary medicine Faculty, Tikrit University, Iraq. Animals were kept under appropriate climatic conditions, 24−27 °C with 12:12 cycle of light and dark, and fed with a good quality pellet diet. The experiment was approved and permitted for conduction by the Institutional Committee of Animal Ethics, Faculty of Vetrinary medicine, Tikrit University. The rats were randomly divided into four groups of three rats per group as follows: group I (negative control) administered 1 ml/kg distilled water; group II administered 5 mg/kg diazepam dissolved in distilled water; group III administered 10 mg/kg diazepam dissolved in distilled water.

Rat parotid tissue samples were fixed in formalin (10%) and paraffin embedding. Paraffin sections were stained using hematoxylin and eosin.

Statistical Analysis

Data were expressed as means ± SD. Statistical differences between groups were applied using SPSS (version 20). All the data were analyzed by T test, data were considered to be statistically significant at P value < 0.01 and < 0.05.

Histological Study

A. Normal Group

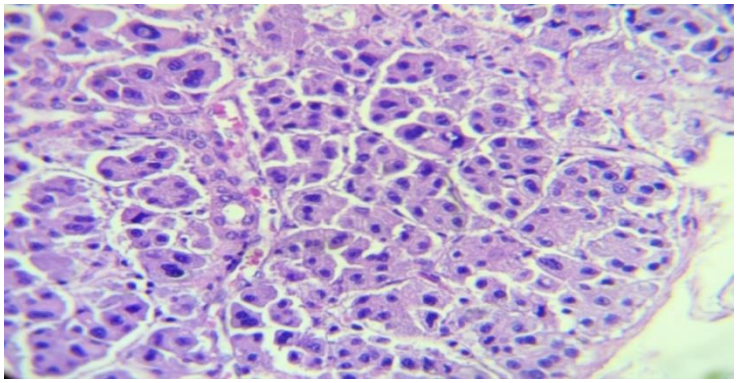

Fig. 1: Parotid gland serous acini (A)capsule of gland (B)intra lobular duct(C)intalobular blood vessels (D)(H&E X40). The Parenchyma of the gland was formed by Acini, each Acinus was lined by pyramidal epithelial cells which had Spherical basophilic nuclei with greish cytoplasm secreting serous media. Each a Cinus was surrounded by delicate connective tissueand trabeculae or septae were encountering a group of acini, intra and inter lobular ducts lined by simple cuboidal cells were present

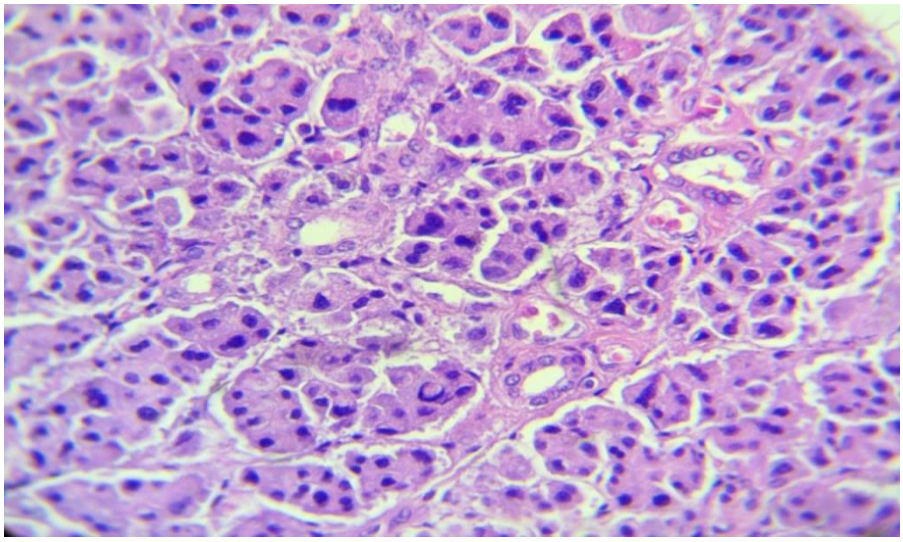

Fig. 2: Parenchyma of Parotid gland. Serous acini (A).Inter lobular duct (B).Intralobular duct (C).(H&E X40).The inter lobular trabeculae had interlobular ducts and blood vesseles with few fibroblasts and lymphocyts

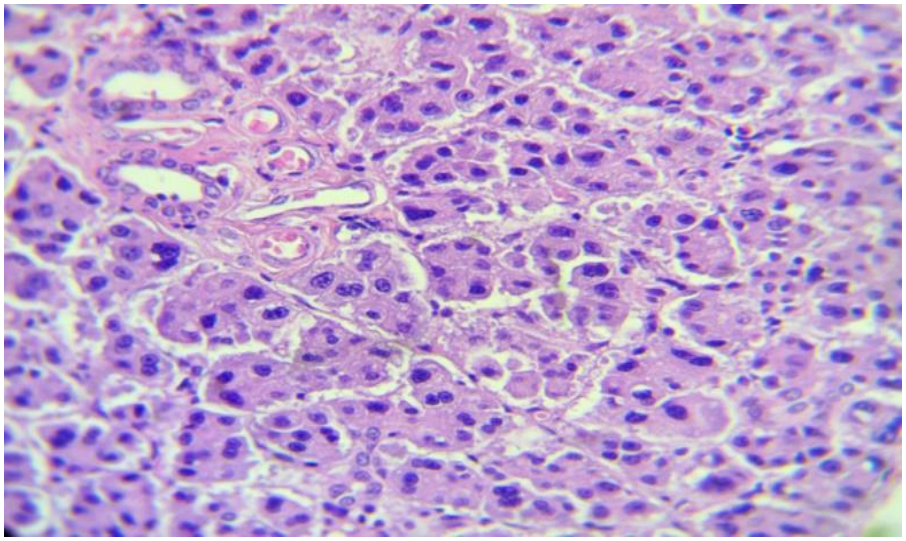

Fig. 3: Parotid gland, serous acini (A).Striated secretion duct (B).Bloodcapillaries (C). Interacinar CT(D). (H&E X40).The inter lobular trabeculae had interlobular ducts and blood vesseles with few fibroblasts and lymphocyts

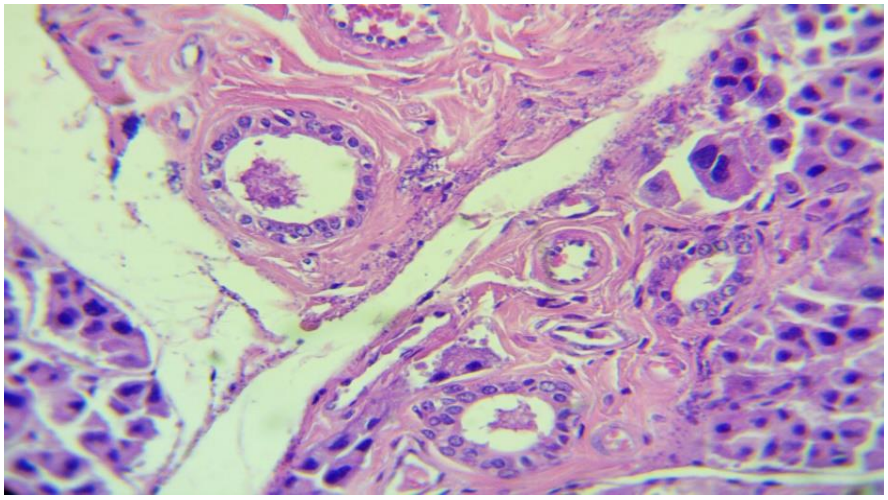

Fig. 4: Serous Acini (A) interlobar duct (B)interlobular duct (C)interlobar blood vessel(D),WBC(E).(H&E X40)

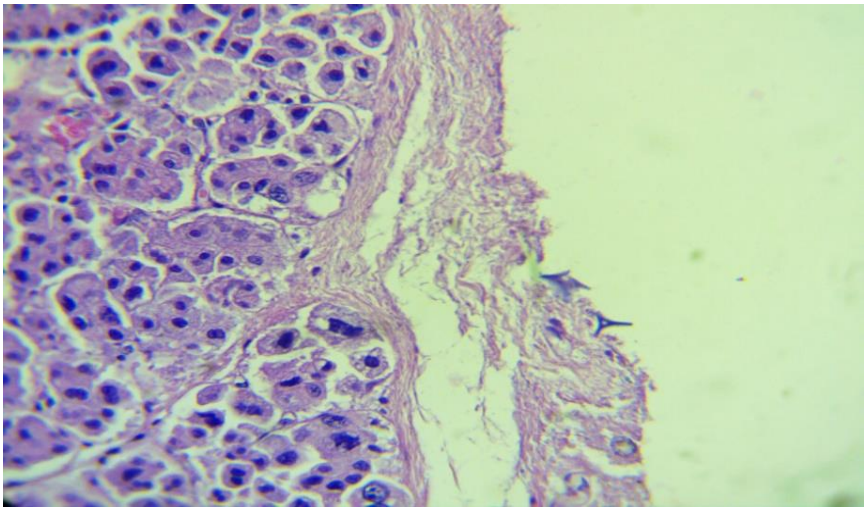

Fig. 5: Capsule of parotid gland (A)Serous acini (B)septa of the the gland (C) interalobular blood vessel (D)(H&E X40)

B. Diazepam Group 5 mg/kg.

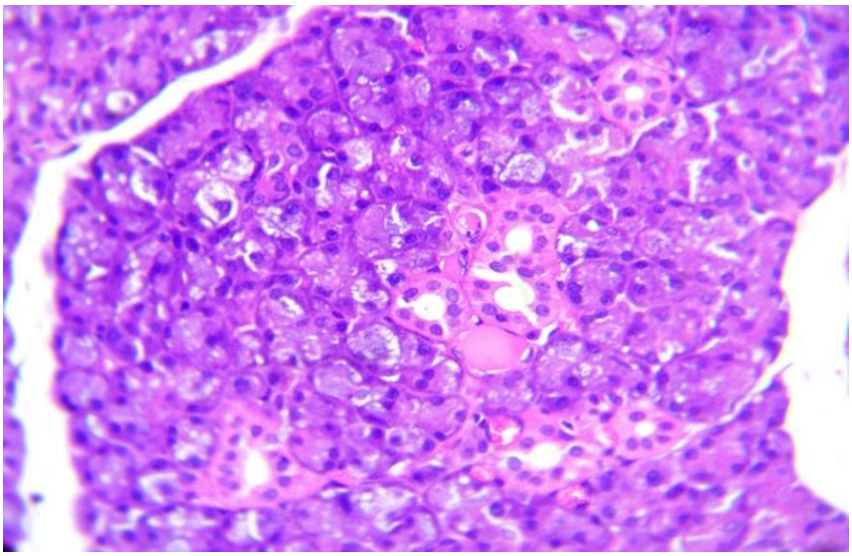

Fig. 6: Most of the serum acini were containing degenerated cells.congested blood vessels around the intralobular ducts

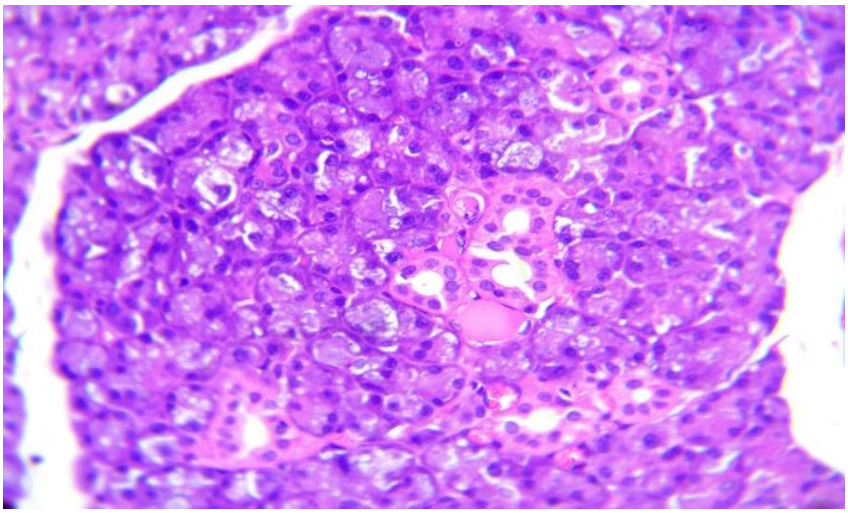

Fig. 7: Vascular degeneration of serous Acinor cells (A).Blood hemolysis (B..Intralobular ducts (C).(H&E X40)

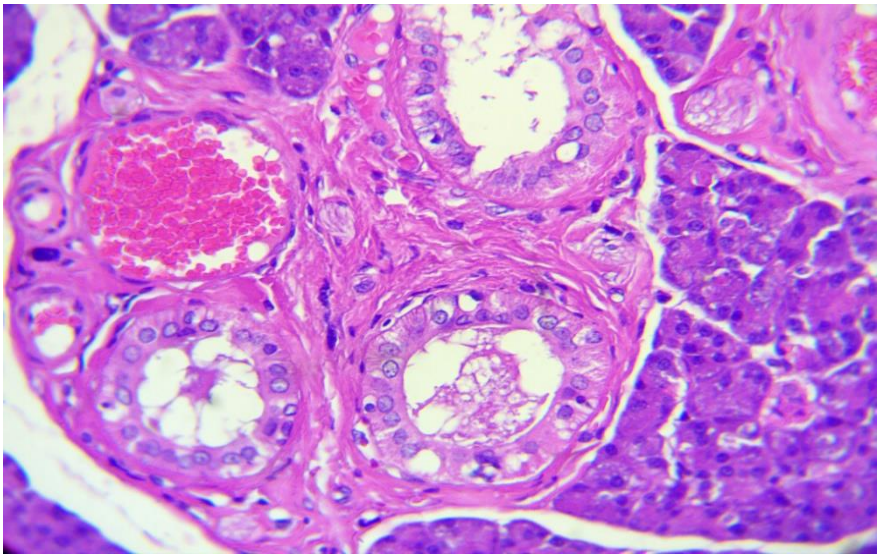

Fig. 8: Interlobar duct (A).decongestion of epithelial cells (B).Lining the ducts enzymal secretion (C).hyperaemia of blood in the interlobor B.V.(D).Macrophages(E)(H&E X40)

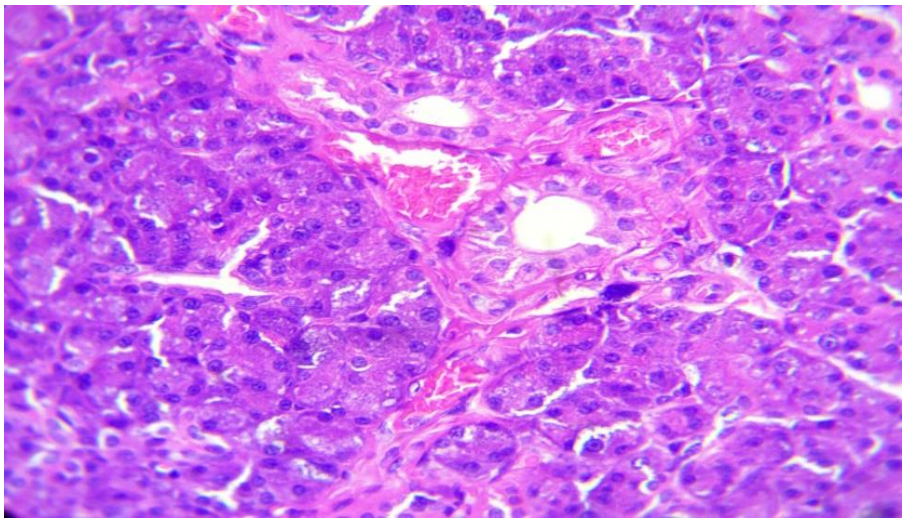

Fig. 9: Crowding of serous acini with cellular degeneration and pyknosis nuclei (A).Blood congestion (B)Striated ducts (C).(H&E X40)

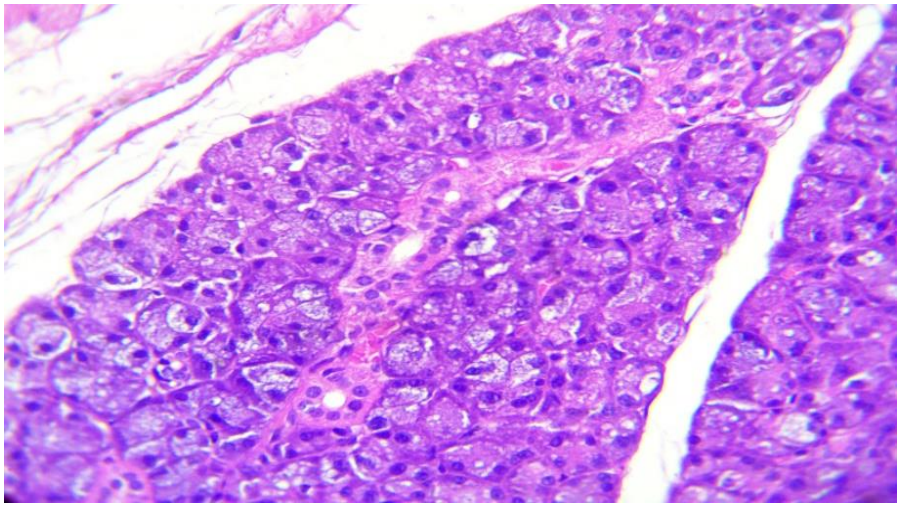

Fig. 10: Degeneration of serous acinir cells with pyknotic nucli (A).Intralobular duct(B).WBC(C)Detachment of capsule (D).(H&E X40)

C. Diazepam Group 10 mg/kg

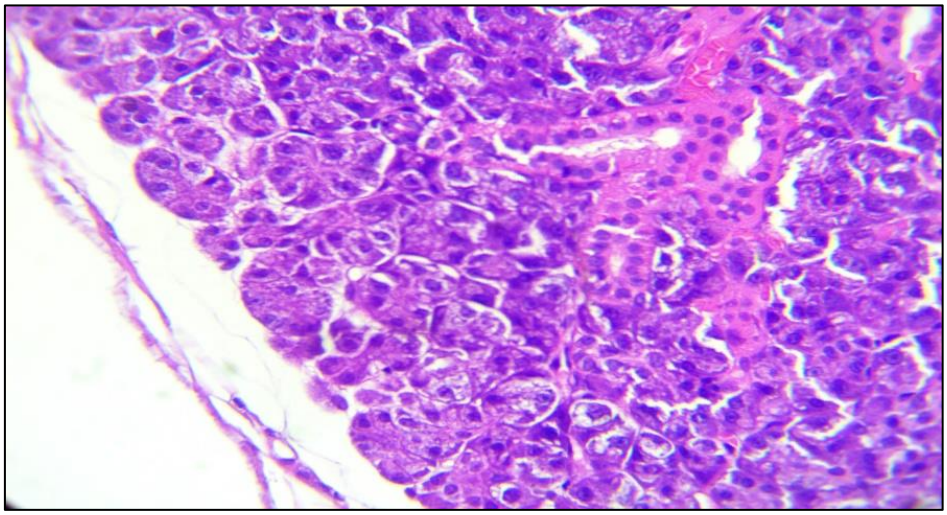

Fig. 11: Glandular detached capsule (A)Atrophy of serous a cini (B)With degeneration of acinar cells, intra lobula duct (C).(H&E X40)

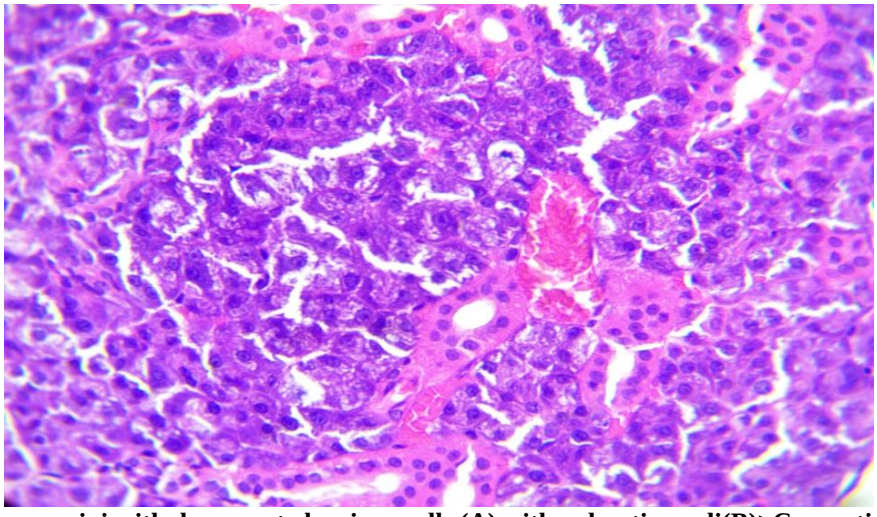

Fig. 12: Serous acini with degenerated a cinar cells (A).with pyknotic nucli(B)>Congestion of blood vessels(C).Intralobular duct(D)(H&E X40)

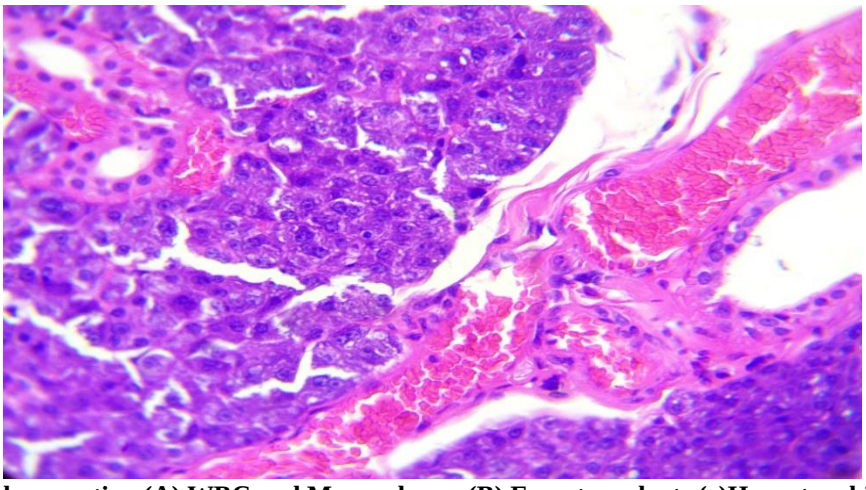

Fig. 13: Sever blood congestion (A).WBC and Macrophages (B) Excretory ducts (c)Hypertrophic serous a cini will ill-defined a cinar cells (D).(H&E X40)

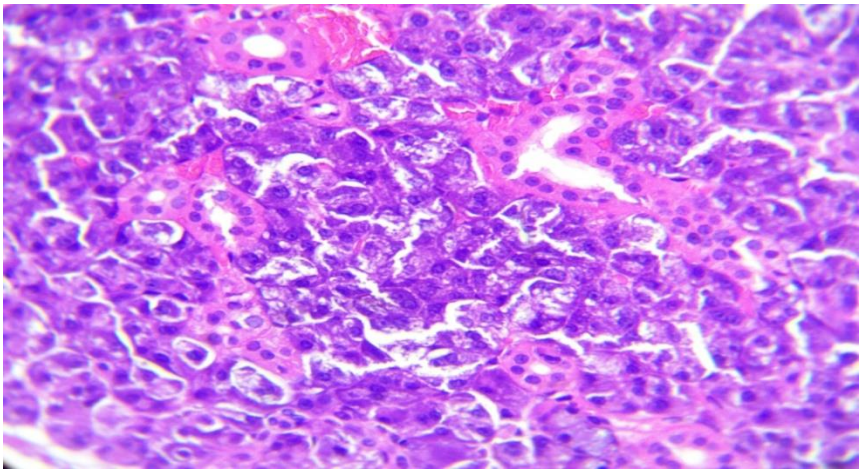

Fig. 14: Etensive degeneration of serous acini (A).Necrotic cells of acini (B).Blood hemorrhage (C).Intercalated ducts (D).Striated duct(E).(H&E X40).

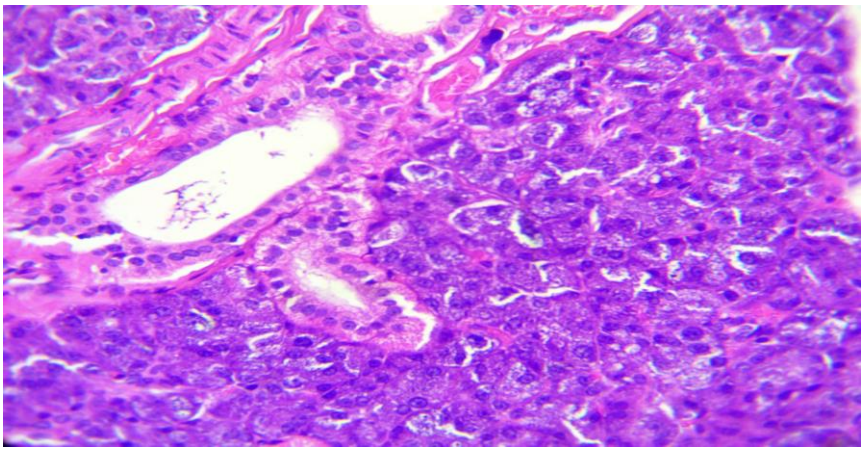

Fig. 15: Aggressive cytoplasmic vacuolization of cell acini (V) is seen, with atrophied acini (T) and pyknosis of nuclei (P) and reduced number of intralobular ducts

The GPX, SOD, and CAT activities significantly decreased ($p < 0.05$) in experimental groups compared to control group (Table 1).

Table 1: The effects of diazepam on GPX, SOD and Catalase in rats

Treatment	GPX(U/L)	SOD(U/L)	Catalase(U/ml)
Sterile Saline	2.4204 ±0.0562	16± 1.29	36.2± 3.156
Diazepam 5mg/kg	1.671±0.3057	11.3±0.732	26.872±2.725
Diazepam 10 mg/kg	0.218±0.0868	6.692±0.958	17.4±3.124

The results indicated that administration of diazepam at doses of 5 and 10mg/kg for 30 days led to a significant increase ($p < 0.05$) in MDA and a noticeable decreased GSH in rats compared to the control groups.

Table 2: The effects of Diazepam on MDA and GSH in rats

Treatment	MDA (µmol/L)	GSH(µmol/L)
Sterile Saline	6.1±1.114	0.3593±0.02447
Diazepam 5mg/kg	8±1.30	0.3039± 0.0391
Diazepam 10 mg/kg	19±1.81	0.13081±0.076

Table 3: The effects of Diazepam on GST,and TAS in rats

Treatment	Glutathione-S-transferase (nmol/mg protein)	TAS (U/ML)
Sterile Saline	22±2.35	1.6± 0.059
Diazepam 5mg/kg	14.27±0.68#	1.32±0.061
Diazepam 10 mg/kg	3.43±0.05 *	1.17±0.052

The GST level of in rat induced with diazepam were significantly lower ($p < 0.05$) than those of the normal control rat. Also the mean of TAC levels were significantly lower in treated group compared to the control groups, as shown in table 3.

DISCUSSION

The salivary gland structure is made up of acinar cells, accessory ducts (intercalated and intralobular), striated ducts, and the principal duct. Its mechanism of action involves the enhancement of Gamma Amino Butyric Acid (GABA), a neurotransmitter by binding to benzodiazepine site on the GABA receptor [19].

They block the actions of the parasympathetic system by inhibiting the effects of acetylcholine on the salivary gland receptors. This results in a dry mouth sensation, probably because the sympathetic portion of the independent nervous system predominates over the "blocked" parasympathetic system the drugs may affect the salivary flow and its composition by interferences in the acinar and duct functions, and by means of alterations in the blood flow of the salivary glands. Besides that, the high serum half-life of Diazepam makes them detectable in saliva for long periods of time probably enhancing their anticholinergic effect on the salivary glands [19]. The diazepam suppress parotid secretion in dose-dependent and non-competitive manner when injected into rats. The inhibitory effect of diazepam is thought to be mediated by both TSPO and CBRs. The effects of diazepam are mediated both by the TSPO (translocator protein), previously known as PBR (peripheral-type benzodiazepine receptor), nd GABAA (γ-aminobutyric acid).

Diazepam act in the in the salivary glands, where both proteins are present. TSPO has been detected in the parotid and submandibular glands in whole-body sections of rat [10-22]. BZDs act to induce reduction in salivary flow through GABAA receptors located in the salivary glands as well as through indirect actions of the CNS on the salivary glands mediating central GABAA receptors. In addition to inhibiting the muscarinic receptors present in the salivary glands, which are responsible for the salivary flow, BZDs also can affect the transport of chloride and the calcium influx, which can lead to fluid secretion. As a result, a reduction in the salivary secretion activity occurs. BZDs can also inhibit β-adrenergic receptors, in turn blocking the release of amylase from rat parotid glands [22-24].

The data presented in this study showed that diazepam increased lipid peroxidation in tissues as expressed by increased tissue levels of MDA, due to increased lipid substrate within the tissue in which can serve as a larger target for oxidation by free radicals resulting from lipid peroxidation of membrane structure, capable of causing cellular injury while interfering with the antioxidant defense system due to the impact of the stress [25, 26]. The findings of the present study data are linked with previous investigations [27, 28]. The deficiency of the increase in MDA level produced by diazepam may be attributed to the depletion of GSH in the interactions of diazepam -induced free radicals with biomembrane and following lipid peroxidation.

Total antioxidant capacity is primarily dependent on antioxidants defence system capabilities and secondly on antioxidants ingested as a part of the diet. Moreover, it also relies on total free radical trapping potential of the body in both normal and oxidative stress conditions. The decreased TAC in oxidative stressed rats can be due to depletion of antioxidants present in the body and increased free radical production [26].

The overall balance between oxidants and GSO antioxidants is reflected by the level of TAC which is understood as the additive action of all the antioxidants present in the selected tissue; hence, it is an integrated factor rather than the ordinary sum of measurable antioxidants, strongly modified by OS [29]. Our study found depletion in TAC in the diazepam treated rats while higher TAC of salivary gland tissue in diazepam -treated groups.

Superoxide dismutase(SOD) is the first detoxification enzyme and most powerful antioxidant that catalyze the conversion of superoxide radicals to H_2O_2 while CAT further converts H_2O_2 to water [30, 31]. The present study, the significant incease in the SOD enzyme activity in the tissues due to facilitates an increase in the production of superoxide which is an important factor portraying oxidative damage which is not healthy for the organ structural integrity [26].

SOD, as a free radical scavenging enzyme along with GSH-Px, and GST, etc. are the first line of defense against oxidative damage. Based on this mechanism, low SOD levels in the treatment group might be related to the antioxidant activity of GSO. The decrease in SOD that occurs, indicates that the antioxidant capacity is low in scavenging ROS or preventing the free radical's generation [32].

Under physiologic situation, GST conjugates with GSH in protecting different tissues from OS effect. GST is groups of large and complex family of proteins that forms the second phase of detoxifying enzyme that conjugates reduced GSH provided by the bile through sulfhydryl group catalysing deactivation of harmful compounds to electrophilic centres on different ranges of substrate toward ensuring their excretion from the cell [33-34]. In this study, activities of tissue GSH were significantly decrease GSH activity could be linked to the adaptive mechanism or defensive

response to toxocity, induction of the apoptosis signalling pathway, removal of OS product, transport of proteins as well as modulation and proliferation of cells.

In response to free radical generation, diazepam administration lowered the levels of antioxidant enzymes CAT in rats, indicating the significant involvement of oxidative stress in diazepam group. Due to the formation of H_2O_2, superoxide anion radicals, and other free radicals.

CONCLUSION

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

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