DIFFERENTIAL EFFECTS OF ALPRAZOLAM AND CLONAZEPAM ON THE IMMUNE SYSTEM AND BLOOD VESSELS OF NON-STRESSED AND STRESSED ADULT MALE ALBINO RATS

BY

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ABSTRACT

Benzodiazepines are one of the most commonly used anxiolytic and anticonvulsant drugs in the world. For nearly all the current benzodiazepines, their toxic effects on different organs have not been fully described. The aim of the current work was to study the immunologic and vascular changes induced by short term chronic administration of alprazolam and clonazepam in non stressed and stressed adult male albino rats. Forty two adult male albino rats were divided into 6 groups (I): (Ia) Negative control, (Ib): Positive control rats received distilled water, (II): Stressed rats, (III): Non stressed rats received daily oral dose of clonazepam (0.5mg/kg), (IV) : Stressed rats received daily oral dose of clonazepam (0.5mg/kg), (V): Non stressed rats received daily oral dose of alprazolam (0.3mg/kg). (VI): Stressed rats received daily oral dose of alprazolam (0.3mg/kg). At the end of the 4th week total leukocyte count (WBCs) and differential count were detected, anti-sheep RBC antibody (Anti-SRBC) titer and interleukin-2 (IL-2) level were assessed, thymus glands, lymph nodes, spleens and abdominal aortae were submitted to histopathological examination. It was found that alprazolam induced a significant increase in the neutrophils count and a significant decrease in the lymphocytes, anti-SRBC titer and IL-2 level with severe depletion of the splenic, thymal and nodal lymphocytes, accompanied by congestion and eosinophilic vasculitis of all tested organs in comparison to clonazepam treated rats. The previous toxic effects were worsened by stress. It was concluded that immune system and blood vessels can be adversely affected by short term chronic administration of alprazolam to a greater extent than clonazepam and these toxic effects are aggravated by stress.

KEYWORDS: *Benzodiazepines, Alprazolam, Clonazepam, Humoral Immunity, Cell Mediated Immunity, Stress, SRBC and IL-2.*

INTRODUCTION

Recent studies have found that stress plays a role in the etiology of many diseases. Stress is generally considered to be immunosuppressive and to increase susceptibility to infections and cancer. Paradoxically, it also exacerbates inflammatory and autoimmune diseases. Although it is well established that stress alters the release of

various hormones and neurotransmitters, the mechanisms by which stress affects immune responses remain elusive (Viswanathan et al., 2005; Salak-Johnson and McGlone, 2007).

Benzodiazepines (BZD) are one of the most commonly used groups of anxiolytic drugs in the world. They are indicated for treatment of generalized anxiety disorders, treatment of panic disorders with or without agoraphobia, sedation, light anesthesia and anterograde amnesia of perioperative events, control of seizures and skeletal muscle relaxation (Iqbal et al., 2002).

Clonazepam, a benzodiazepine derivative, is used for the treatment of epilepsy, psychiatric and neurologic disorders including panic disorders. It has also been utilized in alleviating movement disorders and restless leg syndrome in patients with and-stage renal disease (Brouns and De Deyn 2004; Morishita, 2009).

Alprazolam is one of the most commonly prescribed short acting benzodiazepines in the childbearing period. It has replaced diazepam as the most frequently prescribed anxiolytic drug. This shift in the pattern of drug prescription was justified by the low likelihood of its accumulation (compared with diazepam) and causing sedative effects on multiple doses (Pinna et al., 1997).

All benzodiazepines including clonazepam and alprazolam act by enhancing ≥-aminobutyric acid GABA-ergic neurotransmission through the binding on specific BZD recognition sites, within the GABA (A) receptor-ion channel complex. However, it has been found that BZD also act on peripheral benzodiazepine receptor sites (PBR) or translocator protein 18kDa (TSPO) (Gavish et al., 1999). Evidence for a direct immunomodulatory action for BZD emerged from the recent studies that demonstrated the presence of TSPO on immune/inflammatory cells (De Lima et al., 2010).

The production of antigen-specific antibodies represents a major defense mechanism of humoral immune responses. Data suggest that the primary antibody response to anti sheep RBC antibody (Anti-SRBC) may be one of the most sensitive endpoints available to assess chemical-induced alterations to the immune system. This endpoint has become the cornerstone of several recently established guidelines for assessing the potential immunotoxicity of xenobiotics (Ladics, 2007).

Interleukin 2 (IL-2) is secreted primarily by activated T-lymphocytes and natural killer (NK) cells. It is necessary for regulatory T cell maturation in the thymus and may also sensitize antigen-activated T cells to apoptosis. The importance of IL-2 in supporting the immune response

makes this cytokine and its receptor a prime target for both activation and suppression. Additionally, its inhibition is critical for immunosuppression (Malek, 2003 ; Leo and Hsieh, 2008).

From a toxicological point of view, on reviewing the available published researches, it was noticed that there is little information regarding the influence of clonazepam and alprazolam on different body tissues and cells including the immune and vascular system. Most of the conducted works were focused on their alteration of the immune function rather than the structure. So that, it was thought to be a particular interest to study the toxic effects of these commonly used drugs on these tissues at their maximum therapeutic doses.

AIM OF THE WORK

The aim of the current work was to study the differential immunologic and vascular changes induced by short term chronic administration of clonazepam and alprazolam in non stressed and stressed adult male albino rats.

MATERIAL AND METHODS

1- Drugs:

* **Clonazepam:** It was obtained from Roche, F. Hoffmann-La Roche LTd, Basel, Switzerland in the form of a sterile oral solution contains 2.5mg/ml, supplied with its dropper (1 drop contains 0.1mg clonazepam). On administration, each 0.5 mg of the drug was diluted with 5ml of distilled water.

* **Alprazolam:** in the form of white crystalline powder, was obtained from Amoun Pharmaceutical Industries Co., freshly prepared for oral gavage by dissolving it in distilled water (each 0.3 mg dissolved in 5 ml of distilled water).

2- Kits: Mouse Interleukin-2 (IL-2) ELISA kit of Bioscience, Inc, Catalog Number: 88-7024 was used as an enzyme-linked immunosorbent assay for quantitative measurement of IL-2 in the serum.

3- Sheep RBCs: Were obtained from the laboratory of Zoology Department, Faculty of Veterinary Medicine, Zagazig University.

Experimental Design: Forty two adult male albino rats of average weight 200 gm were brought from the Animal House, Faculty of Medicine, Zagazig University, divided into 6 groups and caged under standardized environmental conditions.

Group I : Twelve rats were equally subdivided into (Ia): Negative control rats, (Ib): Positive control rats, received daily oral dose (1 ml) of distilled water.

Group II : Six stressed rats, were submitted to daily restraint stress at room temperature.

Group III : Six non stressed rats were given daily oral dose of Clonazepam (0.5mg/kg) (Paget, and Barnes, 1964), for 4 weeks.

Group IV : Six stressed rats were given daily oral dose of Clonazepam (0.5mg/kg), for 4 weeks.

Group V : Six non stressed rats were given daily oral dose of Alprazolam (0.3mg/kg) (Paget, and Barnes, 1964), for 4 weeks.

Group VI : Six stressed rats were given daily oral dose of Alprazolam (0.3mg/kg) for 4 weeks.

Both drugs were used in their maximum therapeutic dose for treatment of generalized anxiety disorders.

At the end of the study, blood samples were collected from retro orbital plexus of the rats (5 ml). Total leukocytic count and differential count were detected and immunological studies were performed by measuring the anti sheep RBC hemaglutination (anti-SRBC) titer and the level of serum Interlukin-2 (IL-2). After that, the rats were sacrificed and the thymus glands, lymph nodes, spleens and abdominal aortae were dissected and submitted to histopathological examination.

* **Methods :**

* **Restraint stress Procedure: It** was done by following the procedure described by Glavin et al., 1994. Stress was applied by placing the animals (without squeezing or compression) in well-ventilated wire mesh restrainers for a single daily session of 2.5 h beginning at 9:00 a.m. This procedure mimics stress that is largely psychological in nature.

* **WBCs count and differential count:** Total leukocyte count was determined by using Coulter T660 hematology analyzer, Beckman Coulter, Inc., USA. Two ml of blood samples were collected in tubes containing 20ul EDTA solution and differential count was done on Leishman stained peripheral blood smear.

* **Humoral Immune Response:** It was assayed by estimating Sheep RBC antibody Titer (SRBC): In this test 1.5 ml of blood was collected, the serum was separated and analyzed for hemagglutination titer according to the method described by Ladics et al., 2000. Two fold dilutions (0.025 mg) of sera were made in the microtiter plates with saline. To each well 0.025 ml of 1% (v/v) SRBC was added. The plates were incubated at 37°C for 1 h and then observed for hemagglutination. The highest dilution giving hemagglutination

was taken as the antibody titer. The antibody titers were expressed in a graded manner, the minimum dilution (1/2) being ranked as 1. The mean ranks of different groups were compared for statistical analysis.

*** Cell mediated Immune Response:**

It was assayed through estimation of the cytokine, Interleukin-2 (IL-2): 1.5 ml of blood samples were left for clotting then centrifuged at 3000 rpm for 10 minutes. Serum samples were separated and kept frozen at - 20°C for determination of interleukin-2 (IL-2) by Enzyme Linked-Immuno-Sorbent Assay (ELISA) according to the method described by Hollander et al., 1998. The data analysis program automatically determined the concentrations of IL-2 in the samples by comparing the absorbency of the samples to the standard curve which demonstrated an inverse relationship between Optical Density (O.D.) and the cytokine concentration. IL-2 concentration was represented as pg/ml.

*** Histopathological Examination:** Paraffin blocks and sections of thymus glands, lymph nodes, spleens and abdominal aortae were prepared, stained with Hematoxyline and Eosin and subjected to routine histological examination by following the method described by Bancroft and Stevens (1996).

*** Statistical Analysis:** SPSS Software program was used. Mean values (M)±SD were calculated. t-test and F test were performed. P value of less than 0.05 was considered to be significant.

RESULTS

At the end of the 4th week of the current study, comparison between negative control rats (group Ia) and positive control rats (group Ib) showed a non significant difference in all tested parameters (P>0.05) Table (1).

Stressed non-treated rats (group II) showed a significant increase (P<0.05) in the mean values of neutrophils as compared to control group (Ib). However, it showed a non significant change regarding the mean values of WBCs, lymphocytes, eosinophils and monocytes, when compared with those of the control group (Ib) (p>0.05). It also showed a significant decrease (p<0.001) in the mean values of anti-SRBC titer and a non significant decrease (p>0.05) in the mean values of IL-2 as compared to the control group, as shown in table (1).

Histopathological examination of the same group (group II) showed minimal changes in the spleens and lymph nodes in the form of slight subcapsular edema as shown in figs. (2-a and 2-b), when compared to the normal architecture of control group (Figs. 1-a and 1-b). The thymuses

showed mild subcapsular edema while the Aortae showed eosinophilic vasculitis (Figs. 2-c and 2-d) as compared to the control group (Ib) (Figs. 1-c and 1-d).

Clonazepam-treated non stressed rats (group III) showed a non significant change (P>0.05) in the mean values of WBCs, lymphocytes, neutrophils, eosinophils and monocytes when compared with those of the control group (Ib). It also showed a significant decrease (P<0.001) in the mean values of anti-SRBC titer and a non significant decrease (P>0.05) in the mean value of IL-2 level as compared to the control group (Ib) Table (1).

Comparison between the clonazepam-treated non stressed rats (group III) and the stressed non treated rats (group II) showed a non significant change (P>0.05) in the mean values of all tested immunologic parameters except that of the neutrophils. A significant increase (P<0.05) in the mean values of neutrophils was observed when compared with that of group II as shown in table (2).

The observed changed in the tested organs of the same group (group III) were in the form of congested red pulp of the spleen with normal white pulp, slight depletion of the lymphoid follicles in the lymph nodes specimens (Figs. 3-a and 3-b). Thymus glands showed subcapsular lymphoid follicles (Fig.3-c). Examination of the Aortic tissues revealed multiple plaques protruded inside the arterial lumen. These plaques were composed of smooth muscles and deposition of some foam cells (Fig. 3-d).

Regarding clonazepam-treated stressed rats (group IV), it showed a significant increase (P<0.05) in the mean values of neutrophils. However, the mean values of WBCs, lymphocytes, eosinophils, and monocytes showed a non significant change (P>0.05) when compared with those of the control group (Ib). It also showed a significant decrease (P<0.001) in the mean values of anti-SRBC titer, and IL-2 level as compared to the control group. Tables (1 and 2).

Moreover, it was found that clonazepam treatment in stressed rats (group IV) induced hyaline degeneration of the wall of splenic arterioles with perivascular edema (Fig. 4-a). Lymph nodes examination showed mild depletion of lymphocytes from subcapsular lymphoid follicles (Fig. 4-b). The thymuses showed mild depletion of the cortical lymphoid follicles and medullary congestion (Fig. 4-c). However, the media and intima of the arterial wall had no changes (Fig. 4-d).

Alprazolam-treated non stressed rats (group V) showed a non significant change (P>0.05) in the mean values of WBCs, lymphocytes, neutrophils, eosino-

phils and monocytes when compared with the control rats (Ib). The mean values of anti-SRBC titer and IL-2 level showed a significant decrease P<0.001) as compared to the control group (Ib) Tables (1 and 2).

Spleen sections of the same group (group V) showed depletion of the marginal lymphocytes in the white pulp (Fig. 5-a), and Lymph nodes had slight depletion of the lymphoid follicles (Fig. 5-b). Thymus glands also had mild depletion of the lymphocytes from the lymphoid follicles and mild interstitial edema (Fig.5-c). On examination of Aortic sections, they showed edematous walls, composed of degenerated muscles with endotheliosis (Fig. 5-d).

Comparison between alprazolam-treated stressed rats (group VI) and control rats (Ib), showed a significant decrease (P<0.05) in the mean values of lymphocytes, and a significant increase (P<0.05) in those of neutrophils. In contrast, a non significant change (P>0.05) was detected in the mean values of WBCs, eosinophils and monocytes. The mean values of anti-SRBC and IL-2 showed a significant decrease (P<0.001) when compared with those of the control rats. (Tables 1 and 2).

On comparing the immunologic findings of alprazolam-treated stressed rats (group VI) with those of the stressed non-treated rats (group II), it has been found that alprazolam induced a significant decrease (P<0.001) in the mean values of anti-SRBC titer and IL-2 level. Table (2).

Histopathological findings of the same group (group VI) supported the previous results. Severe depletion of the white pulp lymphocytes and extensive hemorrhages in the red pulp with hemosiderosis and degenerated megakaryocytes were noticed on examination of spleen sections. Moreover, edema and histocytes infiltrations were also observed in the red pulp of the spleens (Figs. 6-a and 6-b). Focal replacement of the lymph nodes subcapsular follicles with reticular cells and macrophages were also detected (Fig.6-c). Furthermore, thymus glands showed marked edema and congestion with few erythrocytes and leukocytes infiltrations (Fig.6-d). Eosinophilic vasculitis was evident on examination of Aortic sections with thick tunica media. Eosinophils and round cells infiltrations in the tunica adventitia were also noticed as shown in fig. (6-e).

When comparing clonazepam-treated non-stressed rats (group III) and alprazolam-treated non stressed rats (group V), a non significant difference (P>0.05) was found in most of the tested parameters. The exception was the anti-SRBC titer, as alprazolam induced a significant decrease (P<0.05) in its mean values when compared to those of clonazepam-treated non stressed rats. Comparison between clonazepam-treated stressed rats (group IV) and

alprazolam-treated stressed rats (group VI), showed that alprazolam induced a significant decrease ($P<0.05$) in the mean values of IL-2 level when compared with those induced by clonazepam. Table (2)

Finally, when observing the differential toxic effects of clonazepam treatment in non stressed rats (group III) and in stressed rats (group IV), a significant decrease ($P<0.05$) in the mean values of anti-SRBC titer and IL-2 level was found in the clonazepam-treated stressed rats. Regarding the differential toxic effects of alprazolam treatment in non stressed rats (group V) and stressed rats (group VI), it was found that alprazolam induced a significant increase ($P<0.05$) in the mean values of neutrophils and a significant decrease in the mean values of anti-SRBC titer and IL-2 level in the stressed rats (group VI) when compared to the non stressed rats (group V) as shown in table (2).

DISCUSSION

Benzodiazepines (BZPs) are widely used drugs as tranquilizers, anticonvulsants and in various other indications as light anesthesia and skeletal muscle relaxation. However, not all benzodiazepines have been tested for their immunotoxic effects (Huemer et al., 2010).

In the current study two commonly prescribed BZPs (clonazepam and alprazolam) have been selected to study their immunologic and vascular toxic potential. Both anti-SRBC titer and IL-2 level were used to assess the humoral and cell mediated immune functions.

IL-2 is necessary for the maturation of thymus lymphocytes, facilitation of immunoglobulins' production made by B cells and the differentiation and proliferation of natural killer cells (Malek, 2003; Waldmann, 2006).

Moreover, it was observed that small numbers of sheep red blood cells (SRBC) markedly augmented the proliferation of T lymphocytes activated by antigens or mitogens. This effect occurred with as few as one SRBC per T lymphocyte and with intact or osmotically lysed red cells. It was also accompanied by increase in the interleukin-2 (Guo and White, 2010).

The results of this study demonstrated a significant immunologic and vascular toxic effects of clonazepam and alprazolam in stressed rather than non-stressed adult male albino rats, after 4 weeks of their administration. These toxic changes were significantly encountered in the immunologic parameters as neutrophils; lymphocytes; anti-SRBC and IL-2 (specially in alprazolam-treated stressed rats), supported by the histopathological findings as depletion of lymphocytes from the spleens, lymph nodes and thymuses, con-

gestion, edema, hemosiderosis, inflammatory cells infiltration, large vessels eosinophilic infiltration, vasculitis, plaques and degeneration.

The previous results were in a harmony with those of Massoco and Palermo-Neto, (2003) and Huemer et al. (2010) who stated that many benzodiazepines have prolonged impairment of cellular immune functions in experimental animals after chronic low administration.

It has been substantiated that stressful stimuli in man as well as in animals lead to suppression of the humoral and cellular components of the immune system. It also found that central nervous system is involved in the regulation of stress-induced immune responsiveness (Yin et al., 2000).

The results of the present study also showed that restraint stress produced an inhibition of SRBC antibody, that was worsen by the administration of either clonazepzm or alprazolam with inhibition IL-2. Kalashnikov et al., (2002) found that, exposure to low doses of BZPs resulted in long-lasting reduction of TNF-alpha, IL-1, IL-6, IL-2 and interferon-gamma.

In 1991, Chang et al. found that alprazolam induced severe inhibitory effects on the proliferative responses of both B- and T-cell. It also reduced production of IL-2 by splenic T-cells, IL-1 and tumor necrosis factor (TNF) by peritoneal macrophages. Moreover, a long lasting depression of lymphocytic proliferation has been described in offsprings of rats that were exposed to either diazepam or clonazepam during pregnancy (Schlumpf et al., 1991).

Regarding the toxic effects occurred to the differential WBC counts, Bautista-Quach et al., (2010) reported a case of pancytopenia following oral administration of 0.25 mg clonazepam twice a day for approximately two weeks.

Lymphocytic depletion, similar to those observed in the current study, was noticed in both red and white pulps of the spleen in rats treated with diazepam (0.1 mg) daily for 4 weeks (Moris, 1991). Moreover, the observed hemosideriosis in spleens of alprazolam-treated stressed rats may be attributed to increased erythrophagia (Pacheco and Santos, 2002). In this context, it was found that diazepam accelerates RBCs destruction via inhibition of Ca^{+2} ATPase on RBCs membrane (Seckin et al., 2007).

Several reports had identified BZPs peripheral type binding sites (PBR) in the endocrine steroidogenic tissues, immune organs and cells, such as macrophages and lymphocytes. Thus, the PBR may be a possible primary target for the immunotoxic effects of BDZ (Righi et al., 1999).

In the current study, immune and vas-

cular toxic effects might be related to cortisol production. West et al., (2001) stated that stimulation of PBR in steroidogenic tissues such as the adrenals increases glucocorticoid production. Glucocorticoid hormones are known for their potent immunosuppressive and anti-inflammatory properties.

Corticosteroids have been shown to promote the immune response during acute stress and to inhibit the immune response during chronic stress (Dhabhar and McEwen, 1997). On the other hand, it was found that restraint stress induces corticosterone secretion (Li et al., 2000).

It was found that elevated endogenous corticosteroids level was linked to reduced spleen cellularity and B cells function in mice (Shi et al., 2003). Moreover, they produced a neutrophilic leukocytosis, lymphopenia and reduced anti-SRBC titer and IL-2 level, a picture similar to that detected in the present study (Anderson et al., 1999; Obmińska-Mrukowicz and Szczypka, 2004).

Alprazolam was tested previously for its effects on corticosteroids production. Chronic administration of alprazolam for 29 days to hamster rats has resulted in increased cortisol and total glucocorticoid levels. It was concluded that alprazolam have a suppressive effect on cortisol production (Arvat et al., 1999).

On the other hand, clonazepam was shown to counteract the effect of stress on cortisol level (Chevassus et al., 2004).

In this context, the cytokine system emerges as a good candidate. Indeed, the production and release of cytokines are known to mediate both inflammatory and immune responses (Wiegers and Reul, 1998), and not only cortisol (Almawi et al., 1996). Schlumpf et al., (1994) reported that PBR stimulation of macrophage and lymphocyte membranes changes the cytokine network.

Interestingly, the action of the PBR ligands seems to be connected with the blockage of voltage dependant Ca^{+2} channels (Ostuni al., 2004). Calcium release appeared to be essential for T cell activation, cytokine synthesis and proliferation. Both increases and decreases in intracellular Ca^{2+} have been linked to apoptosis (Lepple-Wienhues et al., 1999 ; Mason, 1999).

Clonazepam was found to have a strong binding capacity to peripheral benzodiazepine receptors in rat's aortic smooth muscles compared to other benzodiazepines. These binding sites were concentrated in the mitochondria (Cox et al., 1991).

Peripheral benzodiazepine receptors binds with high affinity to cholesterol and transports it across the mitochondrial membrane (Papadopoulos et al. 1997), this

may explain appearance of foam cells and degeneration of smooth muscle observed in the aortae of clonazepam treated rats.

Tanimoto et al., (1999) found that stimulation of the thymus PBR induced apoptosis in the thymocytes. This action was accentuated by dexamethasone administration.

Ekonomopoulou et al., (2010) reported that alprazolam, diazepam and lorazepam exhibit cytogenetic activity in normal human lymphocyte cultures. A possible mutagenic action could explain their immunotoxic effects (Giri and Banerjee, 1996).

Furthermore, an in-vitro study carried out by Saha et al., (2009) concluded that alprazolam can strongly interact with DNA that resulted in conformational changes in the DNA. Similarly, Iakovidou-Kritsi et al., (2009) found that alprazolam induces genotoxicity and cytotoxicity in human lymphocyte cell culture at doses equivalent to oral doses, with a significant increase of Sister Chromatid Exchanges on peripheral human lymphocytes in vivo.

It seems relevant to point out that alprazolam has a triazolo- ring, and a - CH 3 - group that could interact with DNA as an alkylating agent (Brambilla et al., 2007).

In contrast to the current results, Freire-Garabal et al., (1991) found that chronic administration of alprazolam for one month alleviates stress- induced suppression of thymus and spleen cellularity in mice after laparotomy. Covelli et al., (1998) also reported that alprazolam behaves as an immunoenhancer, that enhances the immune cells function.

Thus, clonazepam and alprazolam appeared to modulate immune responsiveness in both non-stressed and stressed animals, albeit in a differential manner and these effects are mediated via the alteration of the in immune system organs' structure (histopathological lesions).

CONCLUSION

It was concluded that immune system and blood vessels can be adversely affected by short term chronic administration of alprazolam to a greater extent than clonazepam, and these toxic effects are aggravated by stress. Further, the results of the present study raise concern regarding safety of benzodiazepines' administration for long periods.

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Table (1): Immunologic parameters in the different study groups after 4 weeks (Anova test).

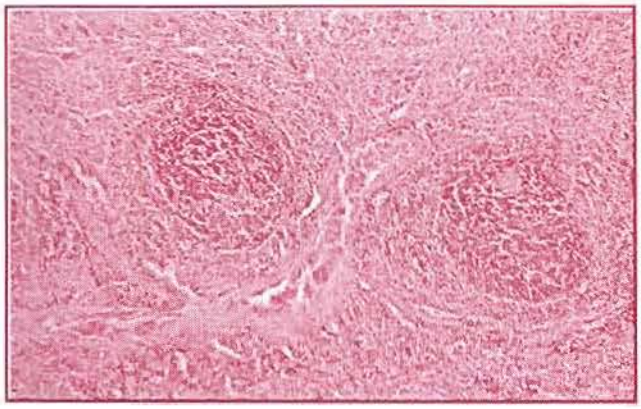
Test		Control rats Group I (Mean ± SD)		Stressed rats (Mean ± SD)	Non stressed + Clonazepam (Mean ± SD)	Stressed + Clonazepam (Mean ± SD)	Non stressed + Alprazolam (Mean ± SD)	Stressed + Alprazolam (Mean ± SD)	F	P
		Group I (a)	Group I (b)	Group II	Group II	Group IV	Group V	Group VI		
Total Leukocyte count ($x10^3/mm^3$)		8.41± 1.10	8.43 ± 0.7	8.39 ± 0.82	8.37 ± 1. 30	8.31 ± 0.61	8.28 ± 0.61	8.21 ± 0.7	0.05	0.90
Differential count ($x10^3/mm^3$)	Lymphocyte	7.21 ± 0.7	6.8 ± 1.9	6.7 ± 1.4	6.8 ± 1.7	6.6 ± 1.3	6.5 ± 1.4	5.1 ±0.5	2.39	0.03*
	Neutrophils	0.90 ± 0.3	0.9 ± 0.6	1.8 ± 0.3	1.0 ± 0.2	1.9 ± 0.8	1.0 ± 0.5	1.8 ± 0.2	5.98	0.001*
	Eosinophils	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.09	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.31	0.92
	Monocytes	0.41 ± 0.1	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.3	0.04	0.99
Anti-SRBC titer		5.87 ± 0.32	5.95 ± 0.3	4.50 ± 0.25	4.00 ± 0.32	3.27 ± 0.25	4.77 ± 0.32	3.18 ± 0.22	83.4	0. 001*
IL-2 (pg/ml)		38.0 ± 6.2	38.1 ± 5.5	36.7± 6.7	35.5± 3.1	32.6 ± 6.7	33.0 ± 5.2	28.4 ± 6.1	11.7	0. 01*

*Significant: $p < 0.05$.; n=6 rats per group.

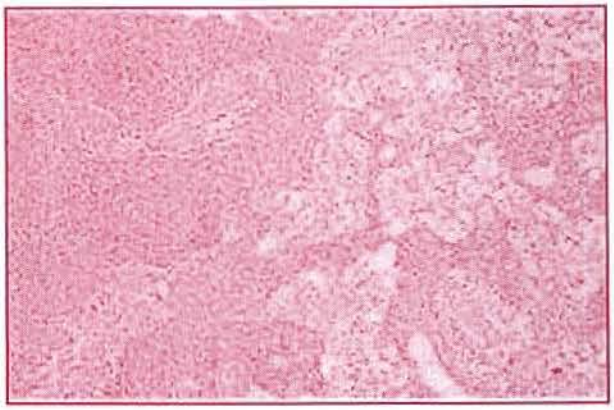
Table (2): Comparison of the mean values for each parameter assessed in the different study groups (LSD test).

	WBC	Lymphocytes	Neutrophils	Eosinophils	Monocytes	Anti-SRBC	IL-2
$P_{1\ (I\ vs.\ II)}$	0.972	0.727	0.01	0.904	0.909	0.001*	0.390
$P_{2\ (I\ vs.\ III)}$	0.950	0.773	0.684	0.945	0.965	0.001*	0.064
$P_{3\ (I\ vs.\ IV)}$	0.854	0.663	0.037*	0.828	0.895	0.001*	0.001*
$P_{4\ (I\ vs.\ V)}$	0.811	0.432	0.768	0.873	0.929	0.001*	0.001*
$P_{5\ (I\ vs.\ VI)}$	0.720	0.047*	0.007*	0.781	0.997	0.001*	0.001*
$P_{6\ (II\ vs.\ III)}$	0.973	0.934	0.019*	0.949	0.892	0.127	0.364
$P_{7\ (II\ vs.\ IV)}$	0.872	0.932	0.801	0.947	0.997	0.001*	0.016*
$P_{8\ (II\ vs.\ V)}$	0.824	0.777	0.053	0.994	0.865	0.396	0.0198*
$P_{9\ (II\ vs.\ VI)}$	0.725	0.189	0.919	0.898	0.926	0.001*	0.001*
$P_{10\ (III\ vs.\ IV)}$	0.917	0.863	0.051	0.873	0.879	0.032*	0.0395*
$P_{11\ (III\ vs.\ V)}$	0.876	0.683	0.977	0.928	0.969	0.033*	0.0518
$P_{12\ (III\ vs.\ VI)}$	0.786	0.1302	0.008*	0.819	0.973	0.016*	0.001*
$P_{13\ (IV\ vs.\ V)}$	0.948	0.859	0.079	0.9217	0.849	0.001*	0.780
$P_{14\ (IV\ vs.\ VI)}$	0.834	0.236	0.847	0.936	0.919	0.752	0.0123*
$P_{15\ (V\ vs.\ VI)}$	0.883	0.226	0.034*	0.856	0.946	0.001*	0.001*

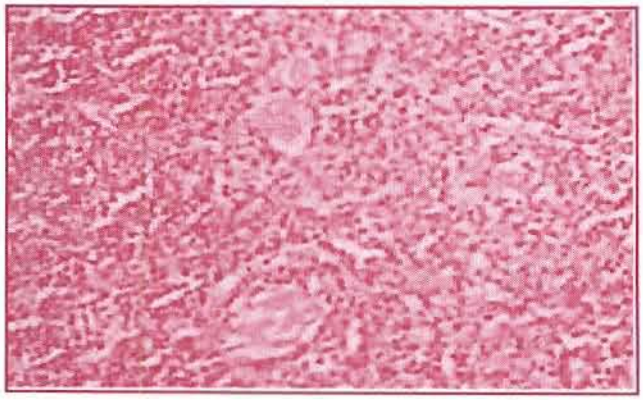
Group I : control; Group II: stressed non treated rats; Group III: non stressed rats received clonazepam;
Group IV: stressed rats received clonazepam; Group V : non stressed rats received alprazolam;
Group VI: stressed rats received alprazolam. ; * Significant: $p < 0.05$.



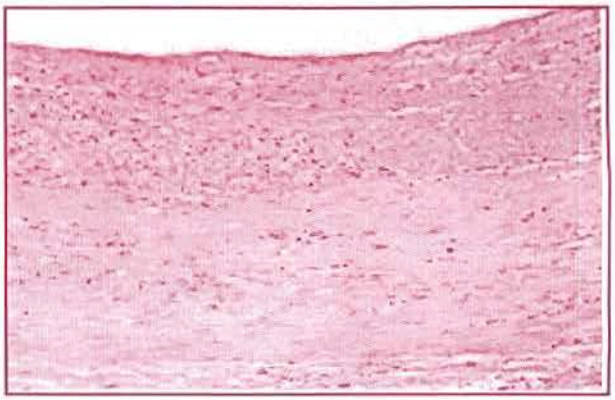
1-a



1-b

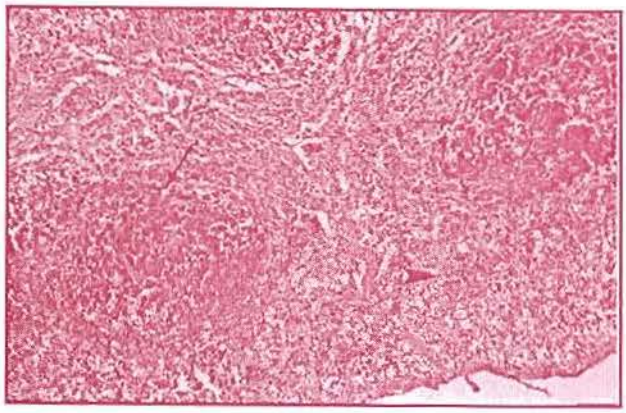


1-c

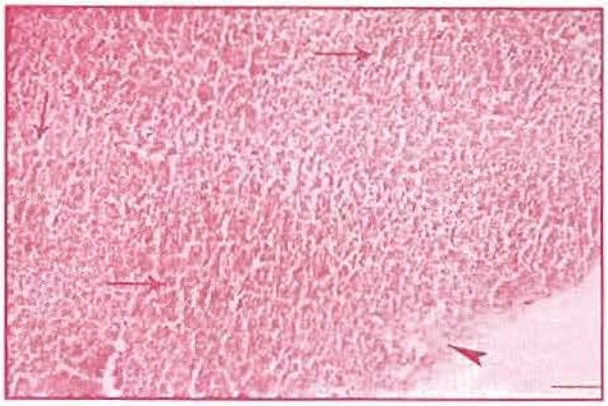


1-d

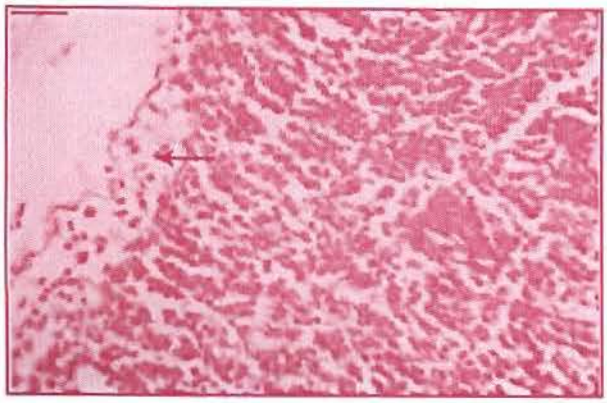
Figure (1): Control rat (group I): a) spleen section showing normal structure (H&E, X 200), b) Lymph node section showing normal structure (H&E, X 200), c) Thymus section showing normal structure (H&E, X 400), d) Large artery section showing normal structure , (H&E, X 200) .



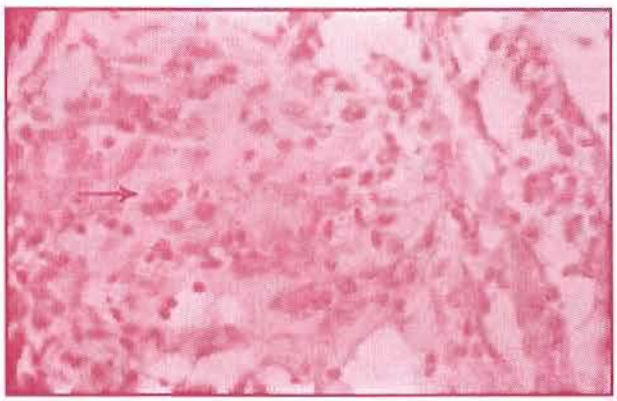
2-a



2-b

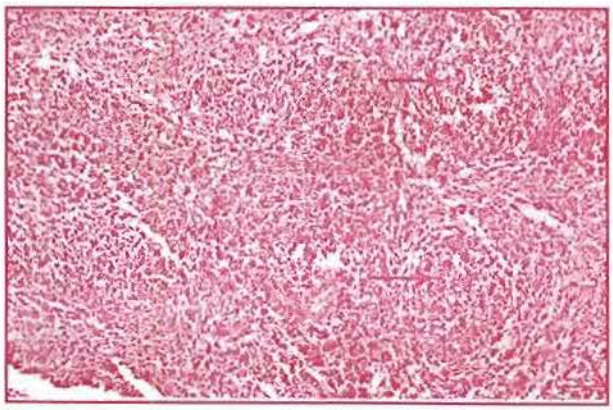


2-c

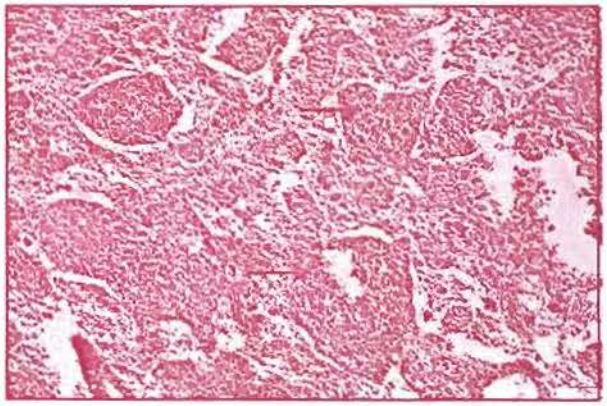


2-d

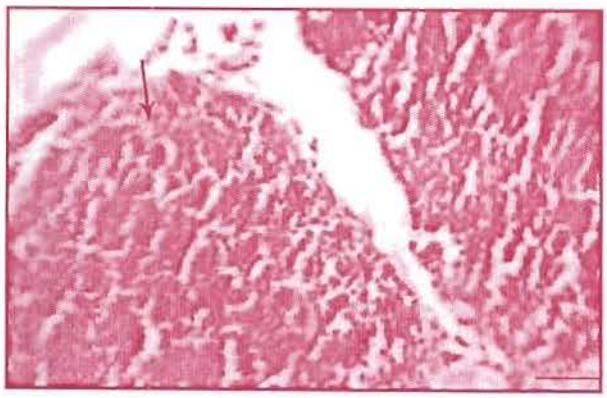
Figure (2): Stressed non treated rat (group II): a) spleen section showing normal white pulp and proliferation of reticular cells (H&E, X 200), b) Lymph node section showing normal lymphoid follicles and mild subcapsular edema(H&E, X 200), c) Thymus section showing mild subcapsular edema (H&E, X 400), d) Large artery section showing eosinophilic vasculitis and eosinophils infiltrations, (H&E, X 400) .



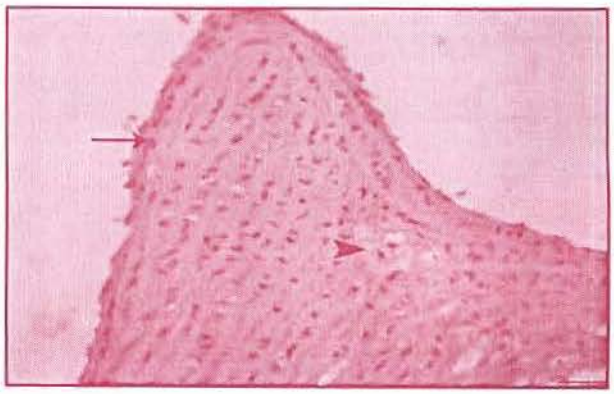
3-a



3-b

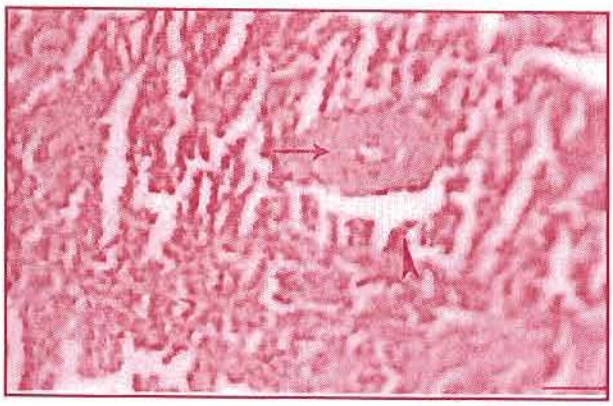


3-c

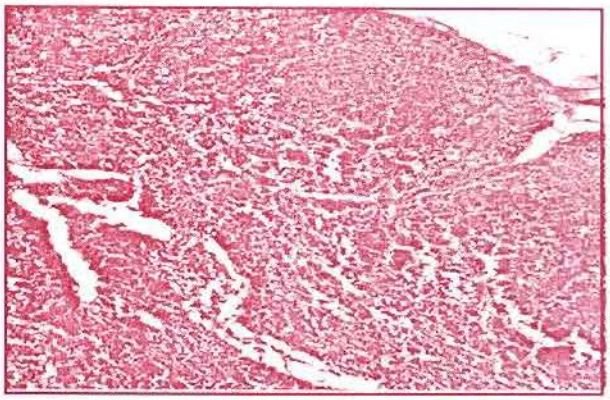


3-d

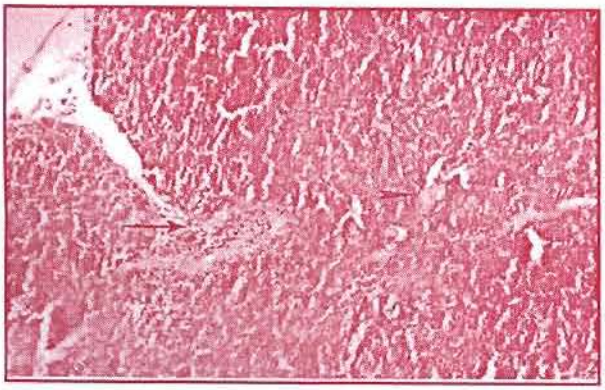
Figure (3): Clonazepam-treated unstressed rat (group III): a) spleen section showing normal white pulp and congested red pulp (H&E, X 200), b) Lymph node section showing mild edema among slightly depleted lymphoid follicles (H&E, X 200), c) Thymus section showing normal subcapsular lymphoid follicles (H&E, X 200), d) Large artery section showing plaque protruded inside the arterial lumen and presented by proliferation of smooth muscles and deposition of some foam cells (H&E, X 200).



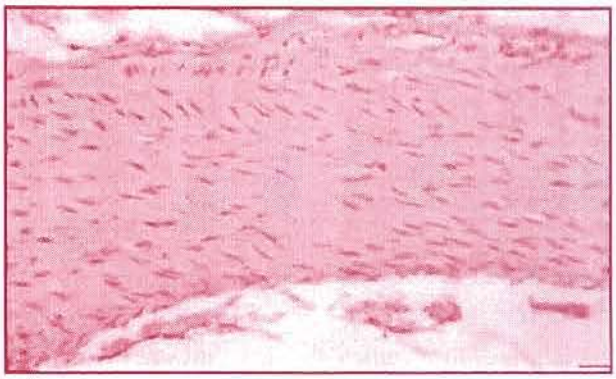
4-a



4-b

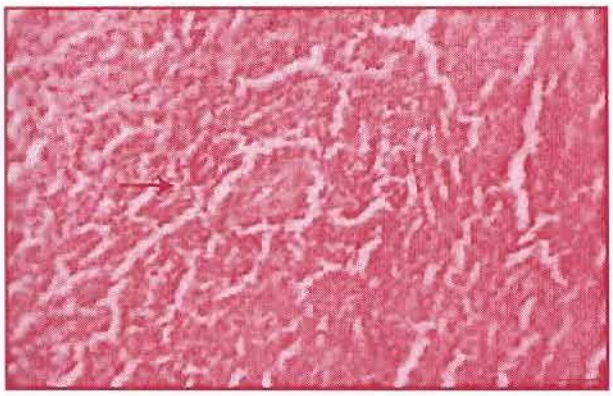


4-c

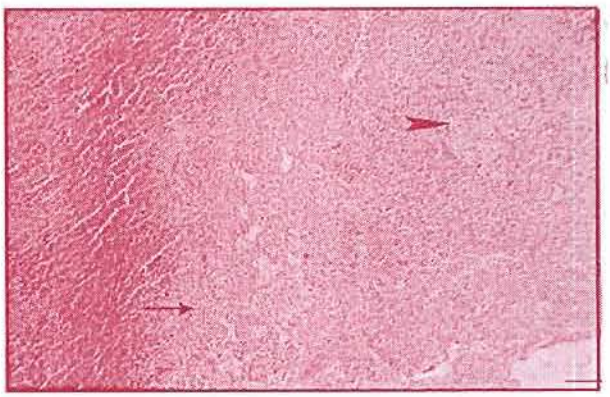


4-d

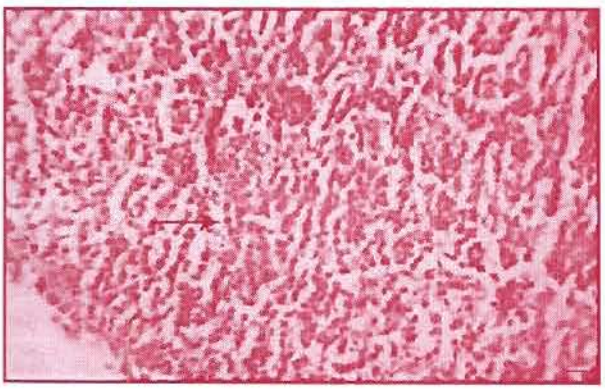
Figure (4): Clonazepam-treated stressed rat (group IV): a) spleen section showing hyaline degeneration of the wall of splenic arteriole and perivascular edema (H&E, X 400), b) Lymph node section showing mild depletion of lymphocytes from subcapsular lymphoid follicles (H&E, X 400), c) Thymus section showing mild depletion of the cortical lymphoid follicles and medullary congestion (H&E, X 200), d) Large artery section showing normal media and intima (H&E, X 200).



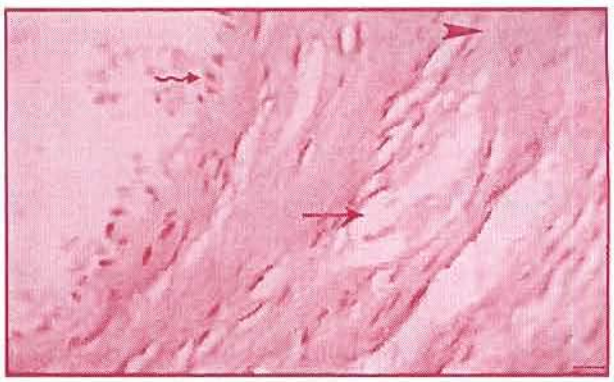
5-a



5-b

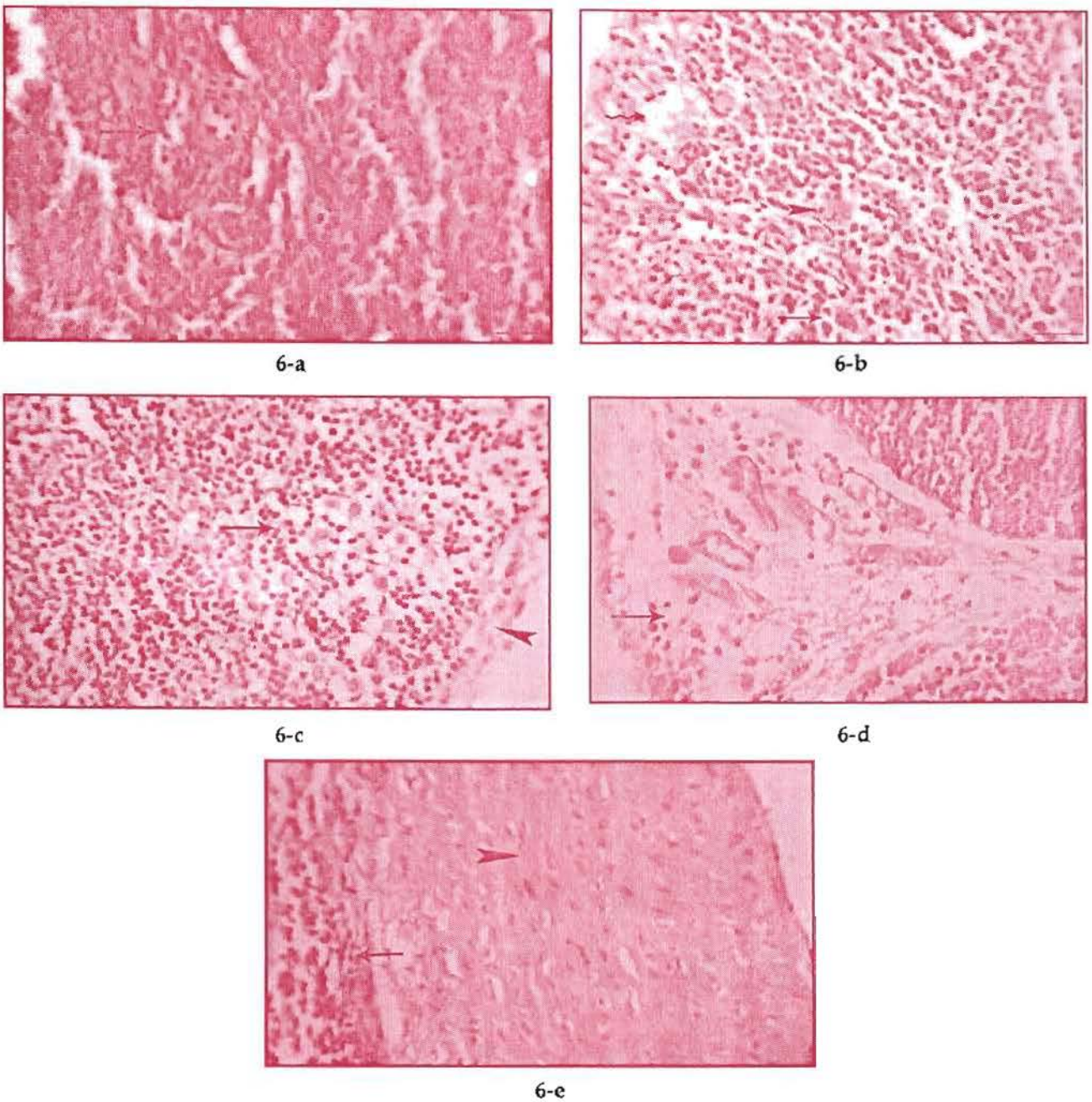


5-c



5-d

Figure (5): Alprazolam-treated unstressed rat (group V): a) spleen section showing depletion of the marginal lymphocytes in the white pulp (H&E, X 400), b) Lymph node section showing mild depletion of lymphocytes from subcapsular lymphoid follicles (H&E, X 200), c) Thymus section showing depletion of the lymphocytes from the lymphoid follicles and mild interstitial edema (H&E, X 200), d) Large artery longitudinal section showing wide space (edema) between degenerated muscles and endotheliosis, (H&E, X 400).



6-a 6-b

6-c 6-d

6-e

Figure (6): Alprazolam-treated stressed rat (group VI): a) Spleen section showing severe depletion of the lymphocytes from the white pulp and extensive hemorrhages in the red pulp (H&E, X 400), b) Spleen section showing hemosiderosis, degenerated megakaryocytes and edema in the red pulp besides histiocytes infiltrations(H&E, X 400), c) Lymph node section showing focal replacement of subcapsular follicles with reticular cells and macrophages (H&E, X 400), d) Thymus section showing showing subcapsular edema, congested capillaries and few erythrocyte and leukocytes infiltrations (H&E, X 200), e) Large artery section showing eosinophilic vasculitis with thick tunica media and eosinophils and round cells infiltrations in the tunica adventitia, (H&E, X 200).

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الآثار المتباينة لعقارى الألبرازولام والكلونازيبام على الجهاز المناعي والأوعية الدموية فى ذكور الجرذان البيضاء البالغة الغير مجهدة عصبيا والمجهدة

المشتركون فى البحث

غادة السيد المسلمى مروة أحمد عباس

أمل حسن عطا* نهلة عبد الغفار رفعت**

أقسام الطب الشرعي والسموم الإكلينيكية، الميكروبيولوجى* و الباثولوجيا**

كلية الطب البشرى وكلية الطب البيطري** -جامعة الزقازيق

مجموعة البنزوديازيبين هي واحدة من أكثر الفئات استخداما في العالم كمهدءات ومزيلات للقلق. بالرغم من ذلك فان الآثار السمية لتلك العقاقير على الجهاز المناعي لم تتم دراستها بطريقة مستفيضة. والهدف من العمل الحالي هو دراسة التغيرات التي تطرأ على الجهاز المناعي والأوعية الدموية، الناجمة عن تناول عقارى الألبرازولام والكلونازيبام لفترة قصيرة المدى فى ذكور الجرذان البيضاء البالغة المجهدة عصبيا والغير مجهده. تم تقسيم اثنين وأربعون من ذكور الجرذان البيضاء البالغة إلى ٦ مجموعات (المجموعة الأولى): (أ) ضابطة سلبية، (ب): ضابطة ايجابية أعطيت الماء المقطر، (المجموعة الثانية): جرذان مجهده عصبيا، (المجموعة الثالثة): جرذان غير مجهده عصبيا أعطيت يوميا عن طريق الفم جرعة من الكلونازيبام (٠ر٥ مجم/كجم) (المجموعة الرابعة): جرذان مجهده عصبيا أعطيت يوميا جرعة عن طريق الفم من الكلونازيبام (٠ر٥ مجم/كجم)، (المجموعة الخامسة): جرذان غير مجهده عصبيا أعطيت يوميا عن طريق الفم جرعة من الألبرازولام (٠ر٣ مجم/كجم) (المجموعة السادسة): جرذان مجهده عصبيا أعطيت يوميا عن طريق الفم جرعة من الألبرازولام (٠ر٣ مجم/كجم). في نهاية الأسبوع الرابع تم قياس ال SRBC ومستوى الانترلوكين - ٢، كما تم فحص أنسجة الغدد الصعترية، والعقد اللمفاوية والطحال والأورطى. وقد وجد أن عقار الألبرازولام أحدث انخفاضا ذو دلالة إحصائية في ال SRBC والانترلوكين - ٢ مع نقص حاد في الخلايا الليمفاوية فى الطحال، العقد الليمفاوية والغدد الصعترية فضلا عن وجود احتقان فى الأورطى بالمقارنة مع الجرذان التي تلقت الكلونازيبام.كما لوحظ أن هذه التغيرات قد تفاقمت في الجرذان المجهدة عصبيا.

الخلاصة: مما سبق يمكن استخلاص أن الجهاز المناعي والأوعية الدموية تتأثر سلبا بالعلاج قصير المدى بعقار الألبرازولام إلى حد أكبر نسبيا من العلاج بعقار الكلونازيبام، وتتفاقم هذه الآثار في حالات التوتر العصبى.