RESEARCH ARTICLE

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HISTOLOGICAL, HISTOCHEMICAL, AND IMMUNOHISTOCHEMICAL STUDIES OF THE CARDIAC MUSCLE OF THE ALBINO RAT UNDER IMMOBILIZATION STRESS AND THE CURATIVE ROLE OF DIAZEPAM

ABSTRACT:
The present study is planned to study the effect of immobilization stress on the cardiac muscle of adult male albino rats and the curative role of diazepam. The study was carried out on 80 albino rats; the animals were divided into eight groups: group 1 served as control rats; group 2 unstressed rats injected intraperitoneally daily with 0.1 mg/kg b.w. diazepam for 30 days; groups 3, 4, and 5 served as immobilized stressed-rats for 30 days (by restricting movement for 2 hr daily at different durations 5, 15, and 30 days, respectively); groups 6, 7, and 8 stressed rats treated daily with 0.1 mg/kg b.w. diazepam for 30 days. The histological study of the cardiac myofibres of the stressed-rats revealed disorganization of the muscle fibres, vacuolation of the sarcoplasm, pyknosis of the nuclei, congestion and dilatation of blood vessels in endomysium. The thickness of collagen fibres gradually increased and became compact dense in the stressed rats till 30 days, and they were more obvious around the blood vessels. The histochemical study demonstrated a marked reduction in the glycogen and protein contents of the cardiac muscle, and was time-dependent. The immunohistochemical study revealed that the rats stressed for different durations manifested the disappearance of the cytoskeletal desmin protein filaments at intercalated discs and Z-lines of the cardiac myofibres. Treatment with diazepam for 30 days to stressed rats demonstrated markedly improvements of the architecture of cardiac myofibres and restoration of the two main chemical components; glycogen and protein contents. Also, restoration of desmin in the cardiac myofibres was elucidated. The results indicated that diazepam is recommended to be used as a curative drug to improve the disturbances in the cardiomyocytes and myofibrils assembly caused under the effect of stress.

KEY WORDS:
Cardiac muscle, Desmin, Diazepam, Histochemistry, Histology, Immunohistochemistry, Rat, Stress

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INTRODUCTION:
Stress is an aversive stimulus, which perturbs the physiological homeostasis, and its impact is reflected on a variety of biological systems. According to the tasks in the life, people can see the degree of stress, more tasks-more stress. Stress responses can be categorized as specific or nonspecific. Specific stressors are typically short-term such as a sudden increase in environmental temperature. Animals typically react to specific stressors by trying to combat the stressor. However, in Long-term or nonspecific stress, animals take measures to adapt to the stressor rather than dealing with it directly (Pacak and Palkovits, 2001).

It has been postulated that stress is involved in pathogenesis of variety of diseases like depression and anxiety. Immobilization (restraint) stress has been commonly used as acute and chronic stress inducers in rats (Bharihoke et al., 2000; Iwa et al., 2006; Kulkarni and Juvekar, 2008). Exposure of striated muscle tissue to intensive and prolonged compression may
pathologically alter its microstructure and lead to loss of muscle fibres cross-striation and infiltration of inflammatory cell (Bosboom et al., 2001; Appell et al., 2004), and also effected on immune system (Brehe and Way, 2008).

Under immobilization stress, the histological changes were elucidated in mammary glands (Soliman, 2006) and induced ultrastructural changes in the colonic mucosa (El-Derieny and Mousa, 2006). Moreover, Erosy et al. (2008) documented that the stomach epithelium of Wister albino rat under stress showed ulceration in some areas, dilatation and degeneration in gastric glandular cells and prominent congestion of the capillaries. Besides, degenerated epithelium and severe vascular congestion were observed in the ileum and colon after the exposure to acute water-avoidance stress for 2 hr daily and chronic stress for 5 days. Gabry et al. (2011) also demonstrated many histopathological changes in the gastric mucosa of rats under immobilization stress for different durations.

El-Refaiy (2010) documented that the immobilization stress of rats for 2 hr daily for different durations (5, 15 and 30 days) caused marked reduction of spermatogenesis, degeneration of germinal epithelium, thickening of basement membrane, haemorrhage and marked increment of collagen fibres. Mazroa and Asker (2010) found ultrastructural changes in zona fasciculate cells of rat adrenal cortex after exposure to high ambient temperature stress. El-Desouki et al. (2011) elucidated the abnormalities in ultrastructure of the three adrenal cortical zonal cells of rat under 2 hr of immobilization stress daily for 30 days.

When an animal first encounters a stressor, the neurogenic system is activated. The neurogenic system is composed of the sympathetic postganglionic neurons and adrenal medullary tissue (Joels et al., 2007). Activation of the neurogenic system leads to marked increases in blood pressure muscle tone, nerve sensitivity, blood sugar and respiration. This is brought about by secretion of the neurogenic amines, epinephrine and norepinephrine. Also, when the hypothalamic-pituitary-adrenal cortical system is activated by stress (Ulrich-Lai et al., 2006), the hypothalamus produces corticotrophin-releasing factor, which in turn stimulates the pituitary to release adrenocorticotropic hormone (ACTH). In most mammals, secretion of ACTH under stress causes the cells of the adrenal cortical tissue to proliferate and to secrete corticosteroids especially cortisol from zona fasciculata (Armario et al., 2008).

Benzodiazepines (BDZs), such as diazepam, are widely used primarily as anxiolytic, muscle relaxant, sedative / hypnotic and anticonvulsant drugs. BDZs reduce stress responses by acting on high-affinity receptor sites present in the central nervous system; these specific binding sites on gama-aminobutyric acid (GABA)-gated chloride channels are called GABA-receptor-chloride-complex (Engel et al., 2007). Nevertheless, besides the central receptors described for BDZs, peripheral-type binding sites have also been identified for them in human stomach, small intestine, colon, liver, lung, thyroid gland, pancreas, breast, prostate, ovary and in mitochondrial membranes (Bribes et al., 2004).

In fact, diazepam treatment has been found to reverse the effect of different stressful stimuli (Lane et al., 1982), to suppress noise induced gastric hypomotility (Gue et al., 1987), to improve the histological and histochemical alterations of testes of immobilized rat (El-Refaiy, 2010) to recover both the histological changes in gastric mucosa (Gabry et al., 2011) and the ultrastructural changes induced by stress in rat adrenal cortex (El-Desouki et al., 2011).

The aim of this work is to study the effect of immobilization stress on the histological, histochemical and on desmin of the cardiac myocytes of male albino rats and the curative role of diazepam.

MATERIAL AND METHODS:

Animals:

Eighty adult male albino rats, each weighing 100 ± 5 g, were used in the present work. The animals were kept under the same natural environmental condition of temperature and photoperiod with free food and water. All procedures and treatments were in accordance with the protocol of National Animal Care and Use Committee and Guidelines for the care and use of experimental animals.

Immobilization stress:

Rats were exposed to stress for 2 hr daily between 10:00 and 12:00 a.m. The animals were individually placed in wire stainless mesh restrainers (5×7×12 cm in dimension) as described by Soliman (2006). This procedure effectively restricted movement of the animals. Control group were housed in normal stainless cages (20 × 25 × 35) Cm. Treatment:

Stressed rats were daily injected intraperitoneally for 30 days with the therapeutic dose of diazepam which is 0.1 mg/kg b.w. according to Paget and Barnes (1964). Diazepam was received from Amoun Pharmaceutical Industries Co. Cairo, Egypt.

Experimental design:

The rats were divided into eight groups, 10 rats each. Group 1: rats received no treatment and served as control; group 2:
unstressed rats injected daily intraperitoneally with a dose of diazepam 0.1mg/kg b.w. for 30 days; groups 3, 4, and 5: rats exposed to stress daily for 5, 15, and 30 days, respectively; groups 6, 7, and 8 rats injected daily intraperitoneally with a diazepam dose of 0.1 mg/kg b.w. for 30 days after 5, 15, and 30 days, respectively of applying the stress.

At the end of the experiment, the rats were sacrificed then the blood sera were collected to measure the level of cortisol. Serum cortisol was determined by using a radioimmunoassay kit (Biochemical, Costa Mesa, CA, USA) and the values were expressed as Ug cortisol /dl serum (Ulrich-Lai et al., 2006).

The cardiac muscle specimens from the left ventricles of the control and treated animals were fixed in 10% neutral formalin, then dehydrated through alcohols, cleared in xylene and then embedded in paraffin wax, then cut to obtain sections of 5 \( \mu \)m thickness which were used for:

1) **Histological study:**
   - Haematoxylin and eosin; H&E (Bancroft and Steven, 1996).
   - Other sections stained with azan to demonstrate the collagen fibres (Humason, 1972).

2) **For histochemical studies,**
   - Periodic acid Schiff’s reagent (Pearse, 1963) was used to demonstrate glycogen content.
   - Bromophenol blue stain (BPB) was used to detect the total protein contents (Mazia et al., 1953).

3) **For immunohistochemical study:**
   Paraffin sections of cardiac muscle and monoclonal antibody (RD301) against desmin were used. Clone, RD301 is a mouse monoclonal IgG2b antibody and reacts exclusively with desmin and is expressed in smooth, cardiac, and striated muscle cells, and was obtained from (Thermo Fisher Scientific Industries). Avidin biotinylated horseradish peroxidase complex ABC technique is applied as detection reagent. Colour reaction was developed by using diaminobenzidine (DAB) and gave a brown colour to desmin. Haematoxylin was used for counterstaining (Hsu et al., 1981).

**RESULTS:**

**Effect of stress on cortisol hormone:**

The cortisol hormone values were measured in the blood sera of rats. The value was 1.35 Ug/dl in the control rats. After 5 days of stress, the hormone levels in the blood sera were increased from 1.35 Ug/dl to 1.53 Ug/dl. The increment of the hormone levels continued after 15 days of stress where it reached 4.535 Ug/dl then post 30 days of stress, the cortisol levels reached 4.03 Ug/dl (Table 1 & Fig.1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Stress for 5 days</th>
<th>Stress for 15 days</th>
<th>Stress for 30 days</th>
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<tr>
<td>Cortisol hormone</td>
<td>1.35</td>
<td>1.53</td>
<td>4.5</td>
<td>4.03</td>
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Fig 1. The correlation between stress and level of cortisol hormone

**Histological observations:**

**Haematoxylin and eosin (H&E):**

In control rats group, the myocardium is striated and arranged in a linear array that branches and anatomizes in a specific pattern giving the appearance of a sheet. The cardiac muscle fibres are joined together by intercalated discs. They contain acidophilic cytoplasm with oval centrally located nuclei. The cardiac muscle fibres are separated by delicate layer of connective tissue with well evidenced myocardial blood capillaries (Fig. 2a). No changes were demonstrated in cardiomyocytes after treatment with diazepam only for 30 days (Fig. 2b).

**Results:**

**Effect of stress on cortisol hormone:**

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The rats group stressed for 5 days revealed variety of histological changes; pale acidophilic cytoplasm of many cardiac myocytes and perinuclear vacuolation were seen. These changes were accompanied with
nuclear peripheralization and pyknosis. Also, congestion of myocardial blood vessels was demonstrated (Fig. 3a). Treatment with diazepam at a dose of 0.1mg/kg b.w. for 30 days of the stressed rats exhibited an improvement in myocardial cells and almost appeared like normal (Fig. 3b).

The rats group stressed for 15 days revealed more obvious alterations, where most of the cardiac muscle fibres demonstrated remarkable disorganization and fragmentation, increment of nuclear peripheralization and pyknosis together with sarcoplasmic vacuolation. Also, marked congestion and dilation of the myocardial blood vessels were displayed (Fig. 4a). The alterations became more prominent in the cardiac muscle fibres of rats stressed for 30 days (Fig. 5a, b).

Treatment with diazepam at a dose of 0.1mg/kg b.w. for 30 days of the rats stressed for 15 & 30 days exhibited an obvious recovery and restoration of the normal architecture of the muscle fibres (Figs. 4b & 6).

Azan stain:
Sections of cardiac muscle fibres of control rats stained with azan revealed normal distribution of blue coloured collagen fibres in the endomysium between the muscle fibres and around the blood vessels (Fig. 7a). Unstressed rats treated with diazepam only for 30 days exhibited normal distribution of collagen fibres as in control ones (Fig. 7b).

In rat groups stressed for 5, 15, and 30 days, there were an excess deposition of collagen fibres in the endomysium of
cardiomyofibres and around the blood vessels (Figs 8a, 9a & 10a). The increment of collagen fibres was time-dependent. After treatment of rats with diazepam, a marked decrease in the distribution of collagen fibres was observed and appeared approximately similar to the control ones, indicating the curative role of diazepam (Figs 8b, 9b, &10b).

Fig. 7. Longitudinal section of the cardiac muscle showing normal dense distribution of collagen fibres in endomysium (arrows): a) a control rat, b) a stressed-rat treated with diazepam for 30 days. Azan, Bar = 6.25µm.

Fig. 8. Longitudinal section of the cardiac muscle of: a) a rat stressed for 5 days showing increase of collagen fibres in endomysium (arrows), b) a stressed-rat for 5 days treated with diazepam showing normal appearance of collagen fibres (arrows) approximately similar to control. Azan, Bar = 6.25 µm.

Fig. 9. Longitudinal section of the cardiac muscle of: a) a rat stressed for 15 days showing high compact dense of collagen fibres around blood vessels and in endomysium of cardiomyocyte, b) a rat stressed for 15 days and treated with diazepam for 30 days showing normal distribution of collagen fibres (arrows). Azan, Bar = 6.25 µm.

Histochemical observations:
Glycogen:

The glycogen distribution in the cardiac muscle of control rats is stained strong pink colour with PAS technique (Fig. 11a) as seen in unstressed rats treated with diazepam for 30 days (Fig.11b). The rats stressed for 5, 15, and 30 days showed gradual marked reduction in the distribution of glycogen materials in the cardiac muscle fibres (Figs12a,13a, &14a). After treatment with diazepam, partial recovery of the glycogen distribution in the muscle fibres was demonstrated (Figs12b, 13b, &14b).

Fig. 10. Longitudinal section of the cardiac muscle of rat stressed for 30 days showing: a) the increment of collagen compact fibres in endomysium and around blood vessel (arrow), b) stressed-rats for 30 days treated with diazepam for 30 days showing normal distribution of collagen fibres between cardiac myocytes (arrows). Azan, Bar = 6.25 µm.

Fig. 11. Longitudinal section of the cardiac muscle showing normal strong distribution of glycogen in the cardiac muscle fibres: a) in a control rat, b) in unstressed rat treated with diazepam for 30 days. PAS, Bar = 6.25µm.
Fig. 12. Longitudinal section of the cardiac muscle of: 
a) a rat stressed for 5 days showing marked 
decrease of glycogen, b) treatment of stressed rat 
(for 5 days) with diazepam for 30 days showing 
partial recovery of glycogen distribution. PAS, Bar 
= 6.25 µm.

Fig. 13. Longitudinal section of the cardiac muscle of: 
a) a rat stressed for 15 days showing an obvious 
reduction of glycogen materials, b) stressed- rat 
(for 15 days) treated with diazepam for 30 days showing 
partial restoration of the glycogen 
distribution. PAS, Bar = 6.25 µm.

Fig. 14. Longitudinal section of the cardiac muscle of: 
a) a rat stressed for 30 days showing the 
depletion of glycogen, b) a rat stressed for 30 
days treated with diazepam for 30 days showing 
partial restoration of the glycogen distribution. 
PAS, Bar = 6.25 µm.

Total protein:

In control and unstressed rat groups 
treated with diazepam, the total protein 
contents appeared as deeply stained granules 
with bromophenol blue in the sarcoplasm and 
nuclei of all the muscle fibres (Fig.15a&b). 
The rats stressed for different durations (5, 
15, and 30 days) showed gradual decrease in 
the protein contents of muscle fibres in the 
sarcoplasm and nuclei (Figs16a, 17a, &18a). 
After treatment with diazepam, the muscle 
fibres restored their proteinic contents and 
appeared rather similar to the control ones 
(Figs16b, 17b, & 18b).

Fig. 15. Longitudinal section of the cardiac muscle 
showing normal strong distribution of total 
proteins content in: a) a control rat, b) 
unstressed rat treated with diazepam for 30 
days. BPB, Bar = 6.25 µm.

Fig. 16. Longitudinal section of the cardiac muscle 
of: a) a rat stressed for 5 days showing a 
marked reduction in the distribution of protein 
contents in the cardiac muscle fibres, b) a rat 
stressed for 5 days and treated with diazepam 
for 30 days showing restoration of normal strong 
protein distribution in the cardiac myofibres. 
BPB, Bar = 6.25 µm.
Immunohistochemical observations:

Immunostaining for desmin showed evident brown colour in the intercalated discs and Z lines of cardiac muscle fibres in the control and unstressed rats treated with diazepam for 30 days (Fig.19a & b). The rats stressed for 5, 15, and 30 days showed an obvious reduction in immunoreactivity of desmin of cardiomyocytes and expressed faint brown reaction in intercalated discs and Z lines (Figs 20a, 21a, and 22a). After treatment of the stressed rats with diazepam for 30 days, a marked increase of immunoreaction intensity for desmin was expressed, indicating the recovery of desmin to a level approximately similar to the control (Figs 20b, 21b, and 22b).
to desmin and expressed with faint brown reaction in intercalated discs (arrows) and Z lines (short thick arrows), b) a rat stressed for 15 days and treated with diazepam for 30 days showing a marked increase of immunopositive reaction to desmin and it appears normal in intercalated discs (arrows) and Z lines (short thick arrows Immunostain, Bar = 6.25 μm.

**DISCUSSION:**

It has been reported that stress involved in the pathogenesis of a variety of diseases like anxiety and depression (Metz *et al.*, 2005; Sevgi *et al.*, 2006; Kulkarni and Juvekar, 2008). Exposure to stress usually affects motor activity and causes pain perception in skeletal muscle (Torres *et al.*, 2001; Appell *et al.*, 2004) and evokes histopathological changes in the gastric mucosa (Gabry *et al.*, 2011)

A familiar stress response in vertebrates is the activation of adrenocortical activity. Acute or chronic stress causes an elevation in corticosterone levels in mammals, hence increased adrenocortical activity is considered as an index of stress response in vertebrates (Nirupama *et al.*, 2010). Cortisol (a natural steroid hormone) is made by the adrenal gland in response to stress such as fasting for prolonged time (Beszczynska, 2005), noise stress (Swami *et al.*, 2007), high ambient temperature (Mazroa and Asker, 2010), and immobilization stress (El-Desouki *et al.*, 2011).

In the present study, the level of the cortisol hormone increased after exposure to different periods (5, 15 and 30 days) of immobilization stress and was time-dependent. In accordance, Gesi *et al.* (2001) reported a marked increase of the cortisol levels in response to noise stressful stimulus that activated the hypothalamic-pituitary-adrenal axis leading to a release of adrenocorticotropic hormone (ACTH). Similarly, immobilization stress response-related disorders resulted in the hypersecretion of both ACTH and corticosterone to a subsequent stressor (Armario *et al.*, 2008). Mazroa and Asker (2010) recorded the increment of cortisol levels in rats after exposure to high ambient temperature. The disturbances may vary by type, intensity, and duration of a stressor, and the strain / sex differentiation of the experimental subjects (Kioukia-Fiouugia *et al.*, 2002). The length of stress period may affect neurological, behavioral, and biochemical parameters, possibly in different ways (Rai *et al.*, 2003).

The present work showed that daily 2 hr immobilized-stress of rats for 5, 10 and 30 days provoked structural changes in the cardiac muscle such as degeneration and disorganization of the muscle fibres, vacuolation of the sarcoplasm, pyknosis of the nuclei and congestion of the blood vessels. The alteration was time-dependent. These results are in agreement with Gesi *et al.* (1999) who observed that after 6 hr of noise exposure; only the atrial myocardial tissue underwent ultrastructural changes, whereas after 12 hr the myocardial damage involved both atria and ventricles. Kušleikaite *et al.* (2004) demonstrated that immobilization stress decreased the relaxation of the smooth muscles and damaged cardiomyocyte ultrastructure as manifested by karyopyknosis, karyorrhexis, desintegration of myofibrils, vacuolization of mitochondria and endoplasmic reticulum.

Moreover, Antona *et al.* (2003) demonstrated that restraint stress produced numerous painful pathologies, such as fibromyalgia, characterized by diffuse muscular pain (hyperalgesia) and/or tenderness (allodynia). With following immobilization, the decrease in muscle power, which is shown as muscle weakness, might depend not only on a quantitative mechanism (loss of mass), but also on qualitative mechanisms, i.e. loss of the intrinsic capacity of muscle fibres to develop force and, in aged muscle, slowing of shortening velocity.

Additionally, acute restraint stress for 6 hr caused severe anxiety like behaviour and impaired locomotor activity as compared with unstressed animals (Kumar *et al.*, 2010). Also, the histopathological changes were induced in stomach, intestine, testis and adrenal gland under immobilization stress in male rats (Gabry *et al.*, 2002; El-Refaiy, 2010; El-Desouki *et al.*, 2011).

Exposure to stress can stimulate numerous pathways leading to increased production of free radicals that contribute to the occurrence of pathological conditions...
Restraint stress may also impair the antioxidant defence system, leading to oxidative damage, by changing the balance between oxidant and antioxidant factors (Ganesan et al., 2011). Malondialdehyde level (a biomarker of lipid peroxidation) was also significantly increased while glutathione level (a biomarker of protective oxidative injury) was significantly decreased in all tissues after exposure to stress (Erosy et al., 2008).

In the current study, collagen fibres increased in endomysium and around blood vessels after exposure to immobilization stress for different times. The enhancement of fibres was time-dependant. In agreement with the present results, Rai et al. (2003 & 2004) and El-Reaiy (2010) found that fibroblasts and collagen fibres in stressed rats were increased and this leads to the thickening of testis basement membrane. Moreover, El-Drieny and Mousa (2006) reported that the colonic mucosa of rats exposed to stress showed features indicating fibrosis as a result of increased collagen synthesis by fibroblasts. El-Desouki et al. (2011) - in their ultrastructural study - reported that the immobilized stressed-rats showed increment of collagen fibres in the adrenal cortex.

The findings of the present study illustrated that the administration of diazepam could improve the histopathological changes and disturbance of collagen fibres resulted under the effect of immobilization stress in the cardiac myocytes. The present results were in agreement with the previous studies reporting that benzodiazepines can reduce or suppress the effects induced by various stress stimuli. In fact, diazepam treatment reduced the ultrastructural alterations in the atrial tissue (Pellegrini et al., 1996), and also reduced disturbances in the brain, kidney, adrenals and heart that were induced by chronic mild stress in rats (Nirmal et al., 2008; Abdel Baky and Ali, 2009). Additionally, ultrastructural changes induced by immobilization stress in rat aden glands (El-Desouki et al., 2011) and in gastric ultrastructure alteration (Nagi., 2012) were modulated by diazepam.

The preventive effect of diazepam is suggested to be attributed to its ability to inhibit the stress-induced activation of HPA-axis and sympathetic stimulation and functional alteration of cell membranes due to steroids (Gehlot et al., 1997). Both central and peripheral BDZ ligands are able to prevent the myocardial damage induced by noise exposure, the extent of this protection depends on the specific drug used and the duration of stress exposure (Gesi et al., 1999). El-Desouki et al. (2011) reported that diazepam administration improved the histological and ultrastructural alterations of the adrenal cortex of immobilization stressed-rats. Moreover, Gabry et al. (2011) reported that diazepam treatment of stressed-rats showed an obvious improvement in the stomach alterations and reduction of collagen fibres after immobilization stress. Antidepressant drugs have also been reported to elevate antioxidant enzyme defence system particularly superoxide enzyme and catalase activity. These antioxidant enzymes raised the level of oxidative defence against stress (Kolla et al., 2005; Kumar et al., 2010).

The histochemical observations in the present work demonstrated that the stressed-rats for different durations showed a significant decrease in glycogen in cardiac myocytes and this was time-dependant. Similarly, Leivo et al. (1998) found that immobilization stress leads to abnormal mitochondria and mitochondrial changes in rabbit skeletal muscles Rosochacki et al. (2000) recorded that immobilization stress caused a decrease of glycogen content in longissimus dorsi muscle by 27% in duroc pigs and by 44% in Pietrain pigs. Nader and Esser (2001) declared that after an acute bout of exercise, significant reduction in muscle glycogen was observed in the tibialis anterior and soleus muscle. Da Silva et al. (2006) and Bosi et al. (2008) found that immobilization significantly reduces the glycogen content in all skeletal muscle.

Such results in eventual structural disturbances and degeneration are probably triggered by a loss of calcium homeostasis (Soares et al., 1993). Brown et al. (1995) explained the reduction of glycogen in muscle may due to the role of reactive oxygen species that cause damage to the mechanisms of carbohydrates synthesis in cells and might be due to inhibition of mitochondrial energy metabolism and the inability of cells to store glycogen and convert lactate and pyruvate to glycogen. Amoroso et al. (2000) illustrated that oxidative stress induces many damaging processes in stress disorders such as disruption of energy pathways, mitochondrial dysfunction and dysregulation of calcium homeostasis.

The present work demonstrated that the immobilization stressed-rats for different durations showed significant depletion in the protein contents in cardiac myocytes and that was time-dependant. In agreement with the present findings, Michelsson et al. (1990) and Graves et al. (1997) demonstrated that several intrinsic tools of fibre wasting could be responsible for muscle atrophy induced by immobilization, contributing to an increased degradation of proteins and a marked reduction in protein synthesis.

The reduction in total protein was also reported by Liu et al. (1996) who showed that the immobilization stress for 8 hr caused...
oxidative damage to protein in rat. Also, significant reduction of proteins in spermatogenic cells of rat testes exposed to oxidative stress was recorded by Sakr and El-Abd (2009) and in rat testes by immobilization stress for different durations (El-Refaiy, 2010). Additionally, the reduction in total protein was demonstrated in rat adrenal cortical cells under immobilization stress ultrastructurally (El-Desouki et al., 2011) and in gastric mucosa (Nagi, 2012).

After treatment of stressed-rats with diazepam in the present work, the glycoprotein and proteinic contents appeared approximately normal as in control ones. Similar results were reported in testes, adrenal gland and gastric mucosa after treatment with diazepam to restraint stressed-rats (El-Refaiy, 2010; El-Desouki et al., 2011; Gabry et al., 2011; Nagi, 2012).

The intermediate filaments (IFs) are one of the three abundant cytoskeletal proteins. The intermediate filaments include cytokeratin, vimentin, desmin, glial fibrillary acidic proteins, neurofilament proteins, nuclear lamins, and nestin (Alberts et al., 1989). They play an important role in the structural integrity and in the transport into and out of the cells. The intermediate filaments provide flexible intracellular scaffolding whose function is to structure cytoplasm and to resist stresses externally applied to the cell (Djabali, 1999; Omary et al., 2004). Cytoskeletal IFs are typically used in animals as an indirect marker of tissue injury (Ananth et al., 2003). Desmin is a 53 kDa of IFs that exhibits a high degree of tissue specificity, its expression being predominantly confined to all types of muscle cells (cardiac, skeletal and smooth muscle). Desmin is localized in the Z-disk region and at the intercalated disk in skeletal and cardiac muscle cells and acts to stabilize sarcomeres in stimulated muscle (Schaart et al., 1989). Paulin and Li (2004) showed that desmin is a muscle-specific protein and a key subunit of the intermediate filament in cardiac, skeletal and smooth muscles. Desmin filaments are mainly located at the periphery of Z-disk of striated muscles and at the dense bodies of smooth muscle cells and they have been postulated to play a critical role in the maintenance of structural and mechanical integrity of the contractile apparatus in muscular tissues.

In the present work, the immunohistochemical results showed an obvious loss of desmin immunostaining in intercalated discs and periphery of Z lines in cardiomyocytes of rats stressed for different durations (5, 15, and 30 days). In accordance, Barash et al. (2002) found loss of desmin immunostaining after a bout of eccentric exercise in the rat tibialis anterior muscles. Such a large-scale change could be the result of depolymerization due to desmin phosphorylation (Inada et al., 1999) or the result of covalent modification such as ADP-ribosylation (Graves et al., 1997), both of which have been observed in other muscle systems.

In the present study, the treatment of stressed-rats with diazepam for 30 days revealed a remarkable improvement and recovery of desmin almost up to the control form in intercalated and Z discs. In agreement with the present findings, El-Baely (2011) found that treatment of rats with diazepam at a therapeutic dose (0.1 mg/kg b.w.) for 30 days improved the adrenal cytoskeletal IFs (cytokeratin and vimentin) of rats stressed for 30 days, and restored them to the normal expression in the adrenal glands (cortex and medulla) and in the gastric mucosa of albino rats (Nagi, 2012).

In conclusion, the immobilization stress may be responsible for the histological, histochemical and immunohistochemical alterations induced in the cardiac muscle of albino rats, and diazepam could improve these alterations.

REFERENCES:


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οϹ΍Ε΍ήϴϐΗ ϪΒΣΎμϳ ΩΎϬΟϹ΍coil ϪοϹ΍Ε΍ήϴϐΗ ϪΒΣΎμϳ ΩΎϬΟϹ΍coil

ΔϴϠϜϴϬϘϧΎϳίϠϟלםόϣΎס驷

΅οϹ΍Ε΍ήϴϐΗ ϪΒΣΎμϳ ΩΎϬΟϹ΍coil ϪοϹ΍Ε΍ήϴϐΗ ϪΒΣΎμϳ ΩΎϬΟϹ΍coil

ΔϠπόϠϟ

Δγ΍έΪϟ΍Ύϣ΃

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ΔϠπόϠϟ

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