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Role of diazepam on stress- related changes in rat liver cytoskeletal Intermediate filaments and CD34 expression.

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ABSTRACT

The present study is planned to investigate the effect of immobilization stress on the cytoskeletal intermediate filaments and CD34 expression in rat liver and the possible curative role of diazepam injected intraperitoneally with therapeutic dose (0.1 mg/ kg b.w.). Sixty adult male albino rats weighing 110 ± 5g were used and divided equally into 6 groups (10 animals / each); group(I) served as control rats, group(II) treated with diazepam, group (III) and (IV) served as stressed- rats (rats were immobilized individually for 2 hrs daily)for two durations 5 and 30 days, respectively; groups (V) and (VI) served as immobilized-stressed rats of the two durations treated with a therapeutic dose of diazepam (0.1 mg/kg bw) for 30 days. The results recorded a significant increase in sera cortisol of the stressed-rats for 5 and 30 days. A reduction of the cytokeratin filaments immunoreactivity in the cell membranes of the hepatocytes was expressed in the two durations. Also, vimentin immunoreaction was decreased in endothelial cells and the connective tissue adjacent the walls of the blood sinusoids. Additionally, the immobilized-stressed rats manifested low CD34 expression in hepatocytes surrounding the central veins; these alterations were time-depended. Treatment of the stressed-rats with diazepam resulted in decreased cortisol levels, marked improvement and restoration of the cytoskeletal cytokeratin and vimentin intermediate filaments as well as the recovery of CD34 expression in the liver. The current results indicated that diazepam is recommended to be used as a curative drug to improve the impairment of the liver cytoskeletal intermediate filaments and CD34 expression caused by stress.

Keywords: Liver, Cytoskeleton, intermediate filaments, CD34, Immobilization stress Diazepam, Rat.

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INTRODUCTION

Stress disturbs physiological homeostasis and plays an important role in the genesis and pathophysiology of different psychological disorders (Garabadu and Krishnamurthy, 2014). Stress induces adreno-medullary response in man to release adrenaline which in turn stimulates receptors on the pituitary gland. It leads to a greater release of adrenocorticotrphic hormone that stimulates the adrenal cortex resulting in further release of cortisol marked increment of the weights of adrenal glands (Lttiyavirah and Sajid, 2014).

Acute stress induced a marked hyperglycemia, decreased liver glycogen content and resulted in tissue injury. Furthermore, repeated exposure to the same stress-stimulus induced a rapid adaptive response that allowed a general protection of cellular integrity (Sanchez *et al.,* 2002). Additionally, Sanchez *et al.* (2007) reported that aggressive encounter (social stressor) caused inflammatory response, and necrotic lesions in liver of mice. Acute restraint stress produces several emotional and autonomic responses. These responses include increased mean arterial pressure and heart rate, skeletal muscle vasodilatation and cutaneous vaso-contraction (Crestani *et al.,* 2009; Alves *et al.,* 2010).

Chronic stress is most prevalent in human beings and has been associated with the development of different pathologies, including cardiovascular, immunological and neurodegenerative diseases and even psychiatric disorders (Bao *et al.,* 2008). Immobilization stress caused increment of free radical production in rat liver and kidney (Rahal *et al.,* 2009), and marked increase of biochemical parameters such as glucose, cholesterol and blood urea nitrogen (Debnath *et al.,* 2011). Acute and chronic stress resulted in increased levels of cortisol, free radicals in brain, stomach and cardiac muscles of rats (Çakir *et al.,* 2010).

The cytoskeleton is composed of microtubules, intermediate filaments "IFs" (cytokeratin, vimentin, desmin, glial fibrillary acidic proteins, neurofilament proteins, nuclear lamins and nestin) and microfilaments. Cytoskeletal filaments play a major role in maintenance of cell shape, cell motility and division, organelles transport and participate in cell-cell & cell-matrix junctions, protection from environmental stresses, cell and intracellular organelles anchorage and muscle contraction (Pallari, 2010; Hassan *et al.,* 2014).

Keratins are found in the epithelial cells and participate in epithelial cell protection from mechanical and non-mechanical stressors. Keratins are used as diagnostic tumor markers as in epithelial malignancies (Karantza, 2011). Liver keratins serve as stress markers. Dynamic increased/decreased keratin site-specific phosphorylation serves as reliable markers for human liver disease progression/regression, and for epithelial cell injury in general (Strand *et al.,* 2007).

Vimentin is present in mesenchymal cells. It is proposed to conistitute a regulatory structure at the receptor enabling efficient signal transmission (Kumar et al., 2007). It is found in almost sarcomas and melanomas but is variable in lymphomas and even some carcinomas (Baharami et al., 2008). It may be coexpressed with cytokeratins in a wide range of carcinomas and other tumors. Increased vimentin expression has been reported in various epithelial cancers including prostate cancer, gastrointestinal tumors, CNS tumors, breast cancer, malignant melanoma, lung cancer and other types of cancers (Satelli and Li, 2011) as well as in colon and upper gastrointestinal tract cancer (Hassan et al., 2014). CD34 is a highly glycosylated sialomucin expressed on a variety of cells, ranging vascular endothelial cells to haematopoietic stem cells, deponding on its glycosylation state. CD34 has been shown to promote proliferation of the haematopoietic progenitor cells and lymphocyte adhesion to vascular endothelium via binding to L-selectin. CD34 is also required for mucosal inflammatory disease development (Gold et al., 2010; Scherberich et al., 2013).

Benzodiazepines (BDZ) such as diazepam are well known to induce antistress properties. BDZ have anxiolytic, sedative, hypnotics, anticonvulsants, skeletal muscle relaxant and amnestic properties (Kulkarni and Juvekar, 2008). BDZ affect the central nervous system through specific binding sites on γ-aminobutyricacid (GABA)-gated chloride channels (Engel et al., 2007). Peripheral-type binding sites for BDZ have also been identified for them in human liver, pancreas, stomach, small intestine, colon, lung, testis, breast, ovary and on the inner and outer mitochondrial membranes (Bribes et al., 2004). Diazepam reduces intra-hippocampus corticosterone concentrations in stressed mice (Béracochéa et al., 2011; Zhao et al., 2012). It improved the histopathological and cytoskeletal intermediate filament alterations of the colonic mucosa of the immobilized-stressed albino rats (El-Desouki *et al.,* 2015a&b).

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As stress is increasing in our life day by day, the present study is planned to investigate the effects of immobilization stress on the cytoskeletal intermediate filaments and CD34 expression in the liver of albino rats and the curative role of diazepam.

MATERIALS AND METHODS

Animals & experimental design: Adult male albino rats weighing 110±5g were used, diet and water were allowed *ad- libitium*, and they were housed in accordance with the Ethics Committee of recommendation of the proper care and use of laboratory animals. The rats were divided into 6 equal groups, 10 animals/each. Group I: served as control; Group II: rats injected daily with diazepam only for 30 days; Groups III & IV: stressed- rats for 5 & 30 days, respectively that were exposed to stress for 2 hrs daily between 9:00 and 11:00 a.m., the animals were placed individually in wire mesh restrainers (5×7×12 cm in dimension) as described by Soliman (2006). This procedure effectively restricted movement of the animal. Groups V&VI: stressed-rats for 5 & 30 days treated with diazepam for 30 days that were injected intraperitoneally (i.p.) with the therapeutic dose of diazepam (0.1 mg/kg bw) according to Paget & Barens (1964) that diluted with distilled water, the treatment was 24 hrs after stress exposure. Diazepam was received from Amoun Pharmaceutical Industries Co. Cairo, Egypt.

Methods: At the end of each experimental period, the blood sera were collected to measure the level of cortisol, and rats were sacrificed after 24 hrs. Serum cortisol was determined by using a radio-immunoassay kit (RIA) (biochemical, Costa Mesa, CA, USA) and the values were expressed as Ug cortisol/dl serum (Ulrich-Lai *et al.,* 2006). The liver specimens were carefully removed, cut into small pieces then fixed in 10 % neutral buffered formalin for 24 hrs and processed to get paraffin sections of 5µm thickness. Monoclonal antibodies against cytokeratin (Pan cytokeratin AE1/AE3) and vimentin (V9) were used (received from Dako Carpinteria, CA 93013 USA.); and polyclonal antibodies against CD34 (were obtained from Thermo Fisher Scientific Industries, Waltham, MA, USA.) were used (Vij *et al.,* 2012). Avidin-biotin immunoperoxidase technique is applied in which a biotinylated secondary antibody reacts with peroxidase conjugated streptavidin molecules. Colour reaction was developed by using diaminobenzidine (DAB) that gave a brown colour. Haematoxylin was used for counterstaining (Toti *et al.,* 2005).

Statistical analysis was performed, statistical significance was determined using one way ANOVA followed by Dunnett's comparison test and cortisol hormone values were expressed as Mean ± SD.

RESULTS

Effect of stress and diazepam on cortisol levels:

The cortisol hormone values were measured in the blood sera of rats. The value was 1.35Ug/dl in control rats, and cortisol hormone of diazepam (Dz) treated rats was 1.38 Ug/dl. After 5 days of immobilization-stress, the hormone levels in the blood sera were increased to 1.53Ug/dl. The increment of the hormone levels continued after 30 days of stress where it reached 4.03Ug/dl. The cortisol levels in stressed rats for 5 days and treated with diazepam for 30 days decreased to reach 1.4Ug/dl, while those stressed for 30 days and treated with diazepam for 30 days decreased to 2.09Ug/dl (El-Desouki *et al.,* 2015 a&b), see Table 1 and Histogram 1.

Table 1: Effect of stress and diazepam treatment on the levels of cortisol hormone

Groups		Cortisol hormone level (ug/dL)	
		Mean	SD
G1 control gp		1.35	0.03
G2 control+Dz		1.38	0.04
G3 Stressed-rats for 5 days		1.53	0.03
G4 Stressed-rats for 30 days		4.03	0.02
G5 Stressed-rats for 5 days +Dz		1.40	0.02
G6 Stressed-rats for 30 days +Dz		2.09	0.03
P-value	G1&G2	0.796	
	G1&G3	0.005*	

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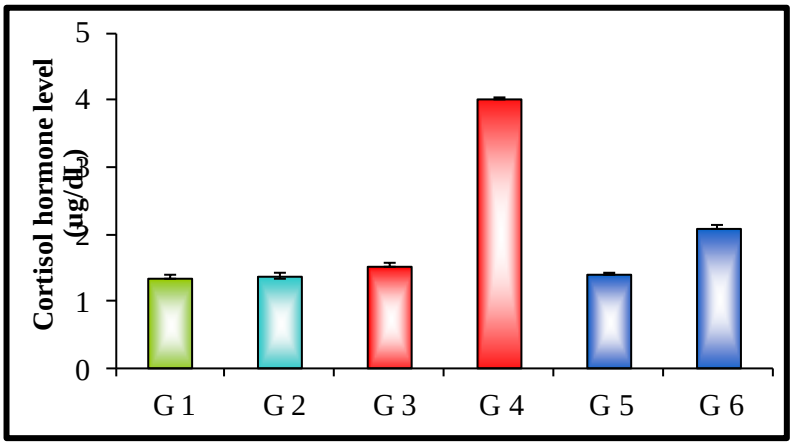
	G1&G4 G3&G5 G4&G6	<0.001* <0.001** <0.001**

All values are mean ± SD, n-10 animals in each group; P*< 0.001 significant increase as compared to control; P**< 0.001 significant decrease as compared to stress.



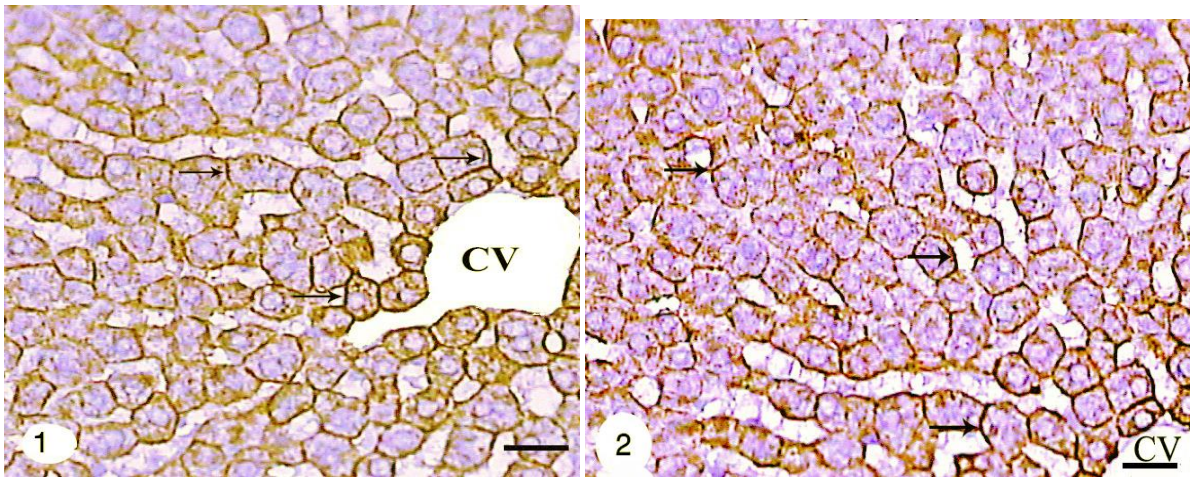
Histogram 1: The correlation between control (G1), control +Dz(G2), stressed – rat groups (G3&G4), diazepam -treated groups (G5&G6) and the levels of cortisol hormone.

Immunohistochemical observations:-

Cytokeratin:-

Liver sections of the control animals expressed normal intense cytokeratin filaments in the cell membranes of hepatocytes and bile duct walls (Fig.1). Rats treated with diazepam (0.1 mg/kg bw) for 30 days expressed normal intense immunoreactivity to cytokeratin similar to the control pattern (Fig. 2)

The stressed-rats for 5 and 30 days expressed markedly reduction of the cytokeratin filaments immunoreactivity in the cell membranes of hepatocytes (Figs.3&4); and after treatment with diazepam (0.1 mg/kg b.w.) for 30 days, an obvious increment of cytokeratin immunoreaction was seen in the cell membranes of hepatocytes (Figs. 5&6). Such reactivity was approximately similar to the control form. The nuclei of such cells exhibited no reaction in all animal groups with monoclonal antibody of cytokeratin.



Figs. (1&2): Sections of the liver of (1) control rat and (2) diazepam treated- control rat for 30 days expressing normal intense cytokeratin filament immunoreactivity in the membranes of hepatocytes (arrows) and in the periphery to central vein (CV). Cytokeratin immunostain, Bar = 12.5 µm.

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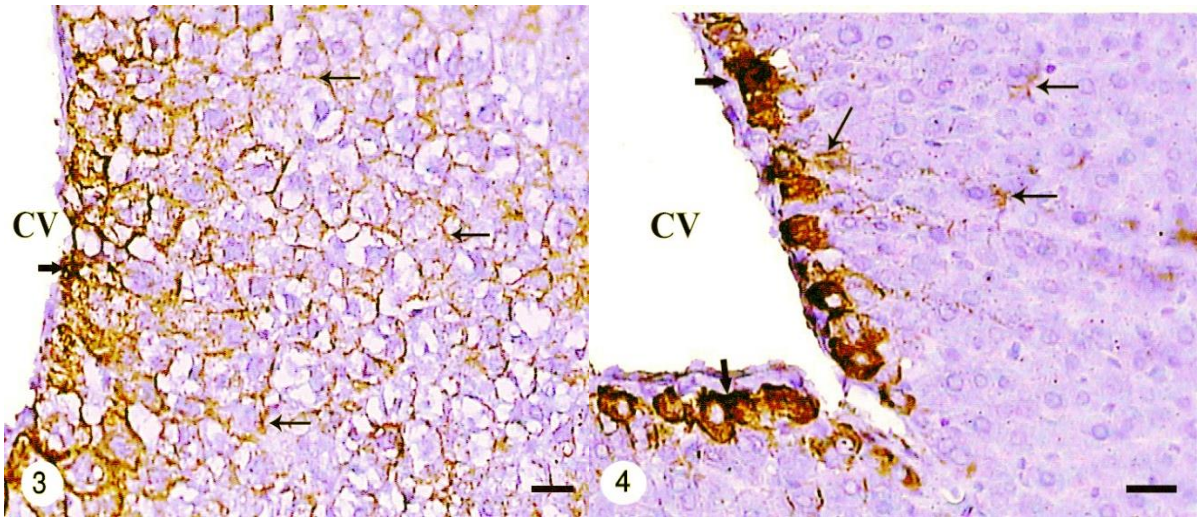
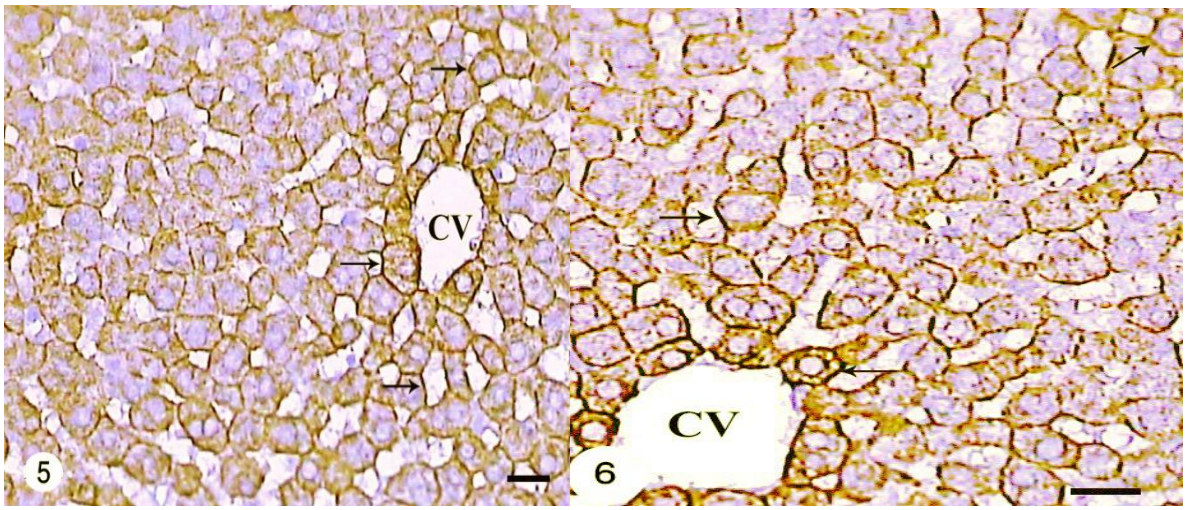


Figs.(3&4): Liver sections of stressed- rats for 5&30 days, respectively expressing (3) an obvious reduction of cytokeratin immunoreactivity in the hepatocytes membranes (arrows) and (4) a marked increase in some hepatocytes periphery to central vein (CV) (thick arrow). Cytokeratin immunostain,Bar= 12.5 µm.



Figs.(5&6): Sections of the liver of stressed- rats for 5&30 days, respectively and treated with diazepam for 30 days expressing a marked increase of cytokeratin immunoreactivity in the hepatocytes membranes (arrows) radiating from the central vein (CV). Cytokeratin immunostain, Bar = 12.5 µm.

Vimentin:-

The liver sections of the control rats expressed the normal intense vimentin filaments immunoreactivity in perisinusoidal cells and in the cells of portal tract stroma as well as in endothelial cells and in the connective tissue adjacent the walls of the central and portal veins, blood sinusoids, and other mesenchymal cells located predominantly in the cytoplasm (Fig.7). The unstressed-rats group treated with a daily dose of 0.1 mg/kg b.w. of diazepam for 30 days expressed vimentin approximately similar to the control form (Fig.8).

The stressed-rats group for 5 & 30 days expressed a reduction of vimentin immunoreactivity to liver sections, and the reduction was time- dependent (Figs.9&10). The treatment of the two groups of stressed-rats with diazepam for 30 days demonstrated a noticeable increase of vimentin immunoreactivity in endothelial cells and in the connective tissue adjacent the walls of the central and portal veins and blood sinusoids (Figs.11&12). Such reactivity was almost similar to the control ones.

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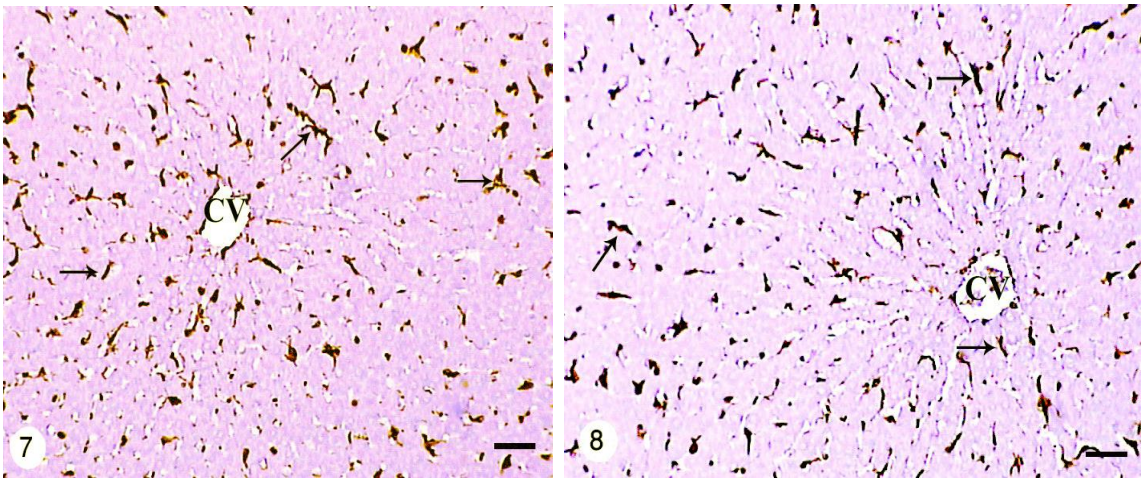
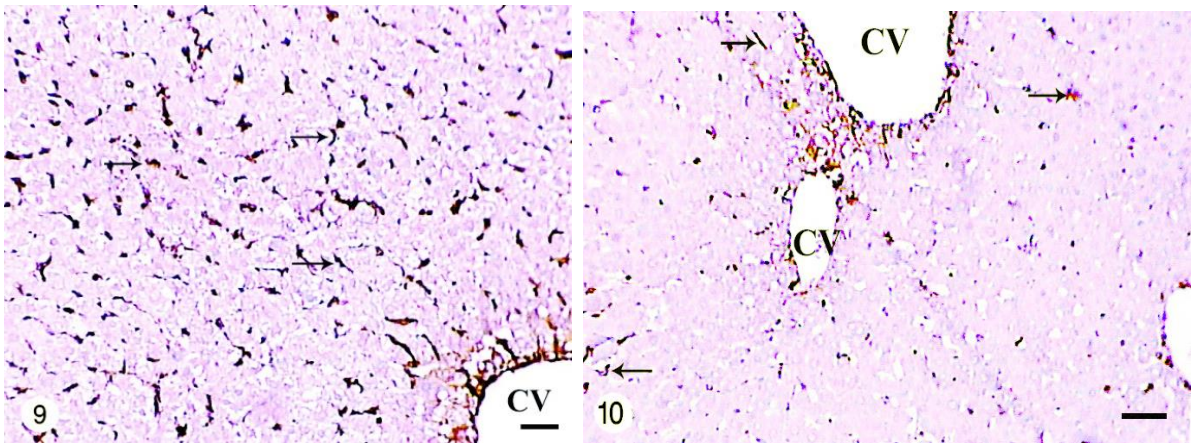
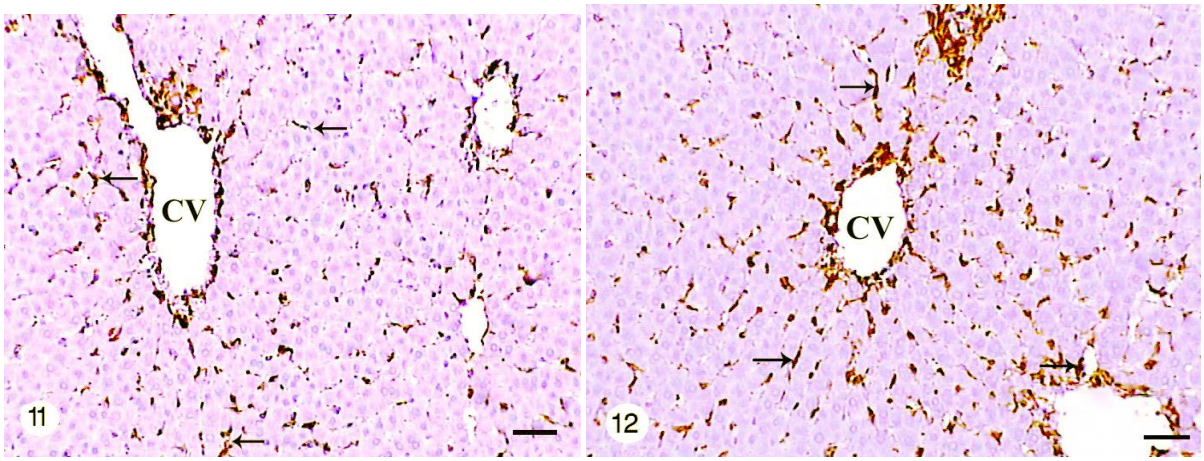


Figs.(7&8): Sections of the liver of (7) control rat and (8) diazepam treated rat for 30 days expressing normal intense vimentin immunoreactivity in endothelial cells and in the connective tissue adjacent the walls of central vein (CV) and blood sinusoids (arrows).Vimentin immunostain, Bar = 12.5 µm.



Figs. (9&10): Sections of the liver of stressed- rats for 5 & 30 days, respectively expressing the decrement of vimentin immunoreactivity in endothelial cells and in the connective tissue adjacent the walls of the blood sinusoids (arrows). Vimentin immunostain, Bar = 12.5 µm.



Figs. (11&12): Sections of the liver of stressed- rats for 5&30 days, respectively and treated with diazepam for 30 days expressing a moderate increase of vimentin immunoreactivity in endothelial cells and in the connective tissue adjacent the walls of central vein (CV) and blood sinusoids (arrows). Vimentin immunostain, Bar = 12.5 µm.

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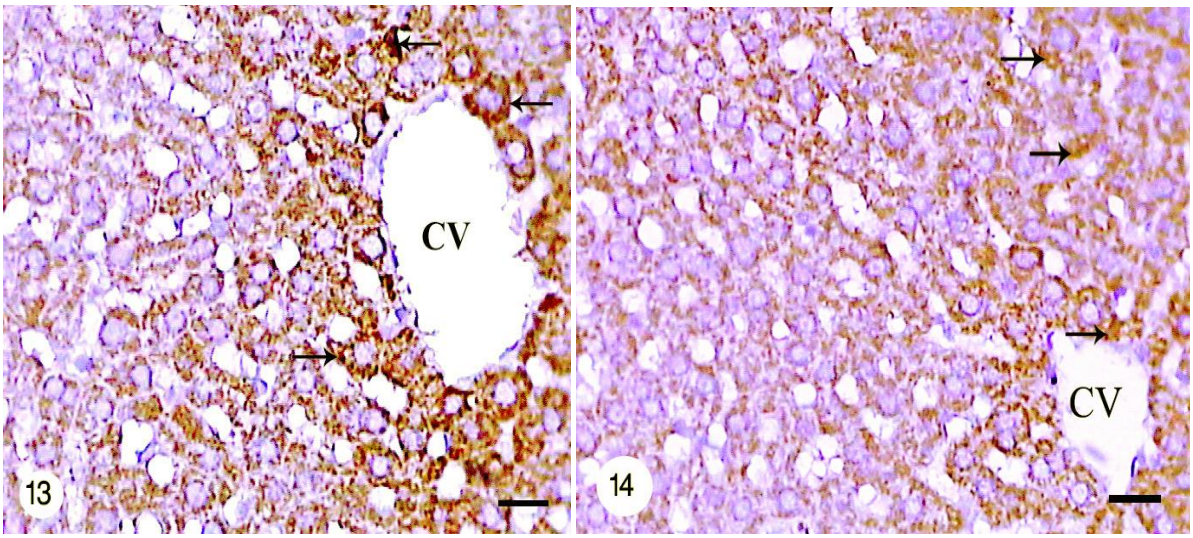
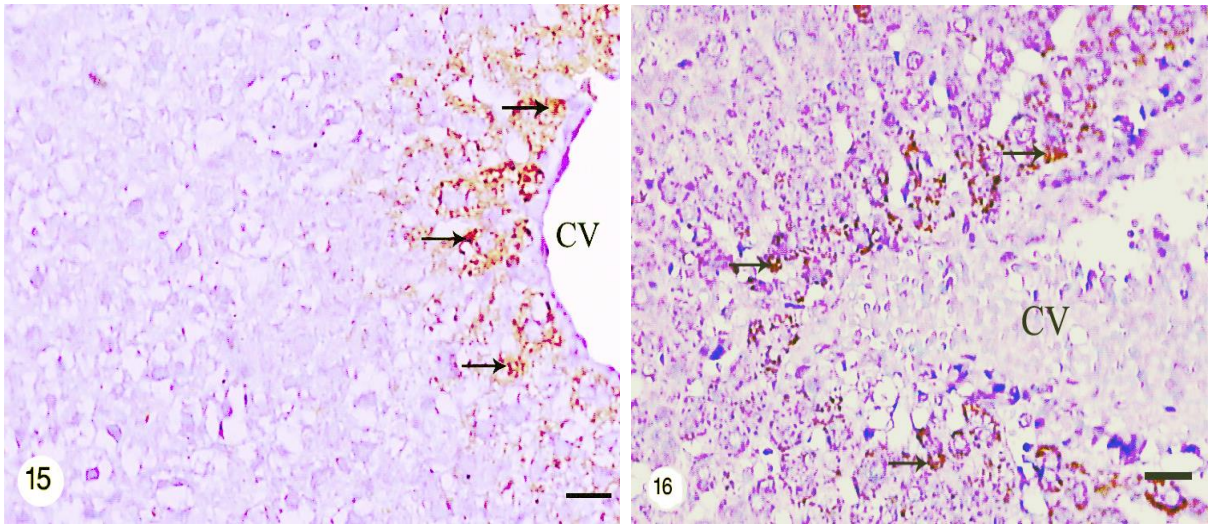
CD34:

The liver sections of control animals expressed the normal moderate CD34 immunoreactivity in hepatocytes surrounding the central veins (Fig.13). The treatment of unstressed- rats with diazepam with a daily dose of 0.1 mg/kg b.w/d. for 30 days revealed normal with no change in CD34 expression pattern (Fig.14).

The immobilized stressed-rats for 5 days illustrated the decrement of CD34 expression in the hepatocytes, and they manifested a scanty CD34 expression in the stressed-rats for 30 days in the hepatocytes (Figs.15&16). After treatment of stressed-rats for 5 and 30 days with diazepam daily at a dose of 0.1 mg/kg b.w./d for 30 days, a moderate increment of CD34 immunoreaction was expressed in the hepatocytes (Figs.17&18), and almost seen similar to the control form.



Figs. (13&14): Liver sections of (13) control rat and (14) diazepam treated- rat for 30 days demonstrating the normal CD34 expression in hepatocytes (arrows) surrounding the central vein (CV). CD34 immunostain, Bar = 12.5 µm.



Figs. (15&16): Sections of the liver of stressed - rats for 5 &30 days, respectively illustrating the decrement of CD34 expression in the hepatocytes (arrows) surrounding the central vein (CV). CD34 immunostain, Bar = 12.5 µm.

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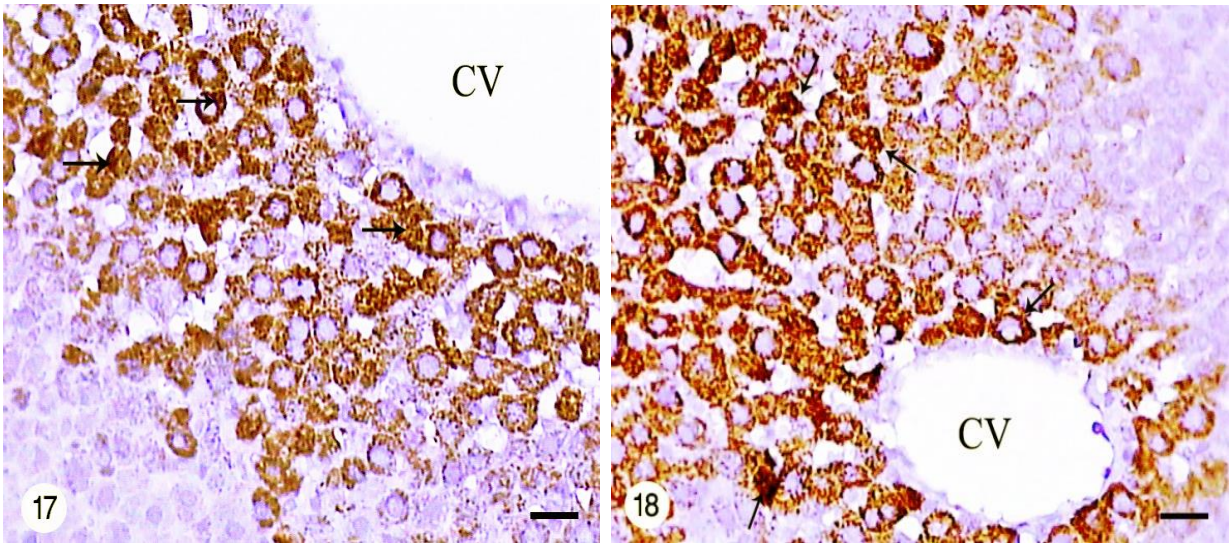

Figs. (17&18): Liver sections of stressed- rats for 5&30 days, respectively treated with diazepam for 30 days expressing marked increment of CD34 immunoreaction in the hepatocytes (arrows) pericentral vein (CV). CD34 immunostain, Bar = 12.5 µm.

DISCUSSION

Stress and anxiety are believed to play a major role in the pathogenesis of many disorders. In the current study, the cortisol hormone levels increased after exposure to different periods of immobilization-stress. The treatment of stressed-rats for 5&30 days with diazepam at a dose 0.1 mg/kg b.w./d for 30 days resulted in the decreased cortisol levels to be normal. In accordance, the results of Çakir et al. (2010) recorded that the acute and chronic stress increased the levels of cortisol in rats). Gabry et al. (2011) declared that the immobilization stress resulted in increment of cortisol level in male albino rats. Chronic immobilization stress increased the plasma corticosterol level and neuroinflammation in mice brain (Perez-Nievas et al., 2011). Also, El-Desouki et al. (2013 & 2015a&b) reported the increment of cortisol hormone levels in immobilized-stressed rats during acute and chronic stress.

The current work revealed that the immobilized-stressed rats for two durations, 5 and 30 days expressed obvious alterations in cytokeratin and vimentin immunoreaction in the liver tissues. In accordance, Flitney et al. (2009) revealed that the moderate shear stress applied to cells for a short period of time caused changes in the phosphorylation which was associated with the building of keratins into thick tonofibrils to enhance the ability of cells to resist mechanical stress.

Keratins of intermediate filaments (IFs) can be considered hepatocyte stress proteins due to their induction upon liver injury and their cytoprotective roles in preventing hepatocytes injury. Alterations in keratin IFs in liver have been demonstrated to render mice more prone to Fas-mediated liver damage (Ku et al., 2003). Cytokeratin (K) 8-null mice have partially distorted hepatic morphology and predisposed to various forms of liver injury (Toivola et al., 2001). The major function of cytokeratin (K8 / K18) in the liver is protection from mechanical and non-mechanical forms of stress (Omary et al., 2002).

Moreover, the K18-mutation in mice resulted in remarkable fragility of hepatocytes and predisposition to various forms of liver injury as they protect hepatocytes from apoptosis (Ku et al., 2007). Mutations in IFs (K8 and K18) predispose the individuals to a wide range of human diseases that generally reflect the tissue-specific expression of the mutant IF gene (Ku et al., 2010). Specific diseases that are associated with IF overexpression in the absence of a mutated IF proteins (e.g Mallory-Denk bodies in alcoholic and nonalcoholic steatohepatitis).This IF inclusion formation related to overexpression of IF which is coupled with an appropriate stress milieu (Pekney and Lane, 2007; Liem and Messing, 2009; Omary et al., 2009).

Vimentin similar to keratins, shears stress deforms vimentin networks in the blood vessels. The importance of vimentin in shear stress relate to focal contacts which became smaller and less able to adhere

to the substratum in vimentin-null cells (Tsuruta and Jones, 2003; Loufrani and Henrion, 2008). Vimentin is crucial for the attachment of lymphocytes to the vascular endothelium and transcellular migration of lymphocytes through endothelial cells (Nieminen et al., 2006). Moreover, in vimentin down regulated cells, the polarization of Golgi complex towards the direction of migration was reported to be disturbed, further indicating the ability of vimentin to affect the Golgi apparatus (Phua et al., 2009). Korita et al. (2010) proposed the co-expression of vimentin and cytokeratin filaments was seen in patients with intrahepatic cholangiocarcinoma.

Use of anti-stress agents such benzodiazepines (diazepam), certain central nervous system stimulants such as amphetamine and caffeine as well as some anabolic steroids are showing significant anti-stress activity against various models of stress. Diazepam-induced antioxidant effects was due to the modulation of the GABA receptors through different benzodiazepine receptor agonists can reduce the oxidative damage produced by acute immobilization and psychological stress (Kumar et al., 2009).

The current results declared diazepam treatment for 30 days to immobilized stress-rats resulted in marked improvement and restoration of the cytoskeletal cytokeratin and vimentin proteins to normal appearance. In agreement, the impairments of cytoskeletal intermediate filaments cytokeratin and vimentin of the rat stomach (El-Desouki et al., 2013) and colonic mucosa (El-Desouki et al., 2015b) by immobilization stress were ameliorated by diazepam treatment. Similarly, diazepam improved the changeable of cytoskeletal intermediate filaments desmin in the cardiomyofibirls of the immobilized-stressed albino rats (El-Desouki et al., 2012). Also, the thyroid gland sections of elderly animals demonstrated an obvious intense immunopositive reaction to vimentin or cytokeratin, and the administration of anti-oxidant vitamin E at a dose 10 mg/kg b.w/day for 60 days illustrated a marked improvement and recovery of vimentin and cytokeratin (El-Desouki et al., 2014).

Concerning to CD34, the present work demonstrated that the immobilized-stressed rats for 5 and 30 days manifested an obvious decrement of CD34 expression in the liver sections, and after treatment with diazepam at a daily dose of 0.1 mg/kg b.w. for 30 days expressed improvement and restoration of CD34 immunoreaction in the liver tissues similar to the normal form. In accordance, CD34 was expressed in the periportal area in normal human liver (Pusztaszeri et al., 2006) and in patients with an abnormal liver functions (Narita et al., 2012). CD34 immunostaining was helpful in the correlation of the sinusoidal capillarization or neo-vascularization to dedifferentiation of the liver tissue during the course of cirrhosis (Gligorijević et al., 2010). CD34-positive stem cells have been shown to induce therapeutic angiogenesis in animal models of myocardial, peripheral, and cerebral ischemia, and in the treatment of heart and vascular disease in human beings (Mackie and Losordo, 2011).

From the present study, diazepam is recommended to be used as a curative drug to improve the cytoskeletal cytokeratin and vimentin intermediate filament alterations as well as CD34 expression in the liver caused under the damaging effects of acute and chronic immobilization stress.

REFERENCES

[1] Alves, F. H.; Crestani, C. C. and Correa, F. M. (2010): The insular cortex modulates cardiovascular responses to acute restraint stress in rats. Brain Res., 1333: 57 - 63.

[2] Baharami, A.; Truong, L. D. and Roj, y. (2008): Undifferentiated tumor: True identity by immunohistochemistry. Arch. Pathol. Lab. Med., 132: 326-348.

[3] Bao, L.; Yao, X. S.; Yau, C. C.; Tsi, D.; Chia, C. S.; Nagai, H. and Kurihara, H. (2008): Protective effects of bilberry (Vacciniummyrtillusl) extract on restraint stress-induced liver damage in mice. J. Agri. Food Chem., 56(17): 7803-7807.

[4] Béracochéa, D.; Tronche, C.; Coutan M.; Dorey, R.; Chauveau, F. and Piérard, C. (2011): Interaction between diazepam and hippocampal corticosterone after acute stress: impact on memory in middle-aged mice. Front. Behav. Neurosci., 5(14):1-9.

[5] Bribes, E.; Carriere, D.; Goubet, C.; Galiegue, S.; Casellas, P. and Simony-Lafontaine, J. (2004): Immunohistochemical assessment of the peripheral benzodiazepine receptor in human tissues. J. Histochem. Cytochem.,52(1): 19 – 28.

[6] Çakir, B.; Kasımay, Ö.; Kolgazi, M.; Ersoy, Y.; Ercan, F. and Yeğen, B.C. (2010): Stress-induced multiple organ damage in rats is ameliorated by the antioxidant and anxiolytic effects of regular exercise.Cell Biochem. Function, 28(6): 469-479.

[7] Crestani, C. C.; Alves, F. H.; Tavares, R. F. and Correa, F. M. (2009): Role of the bed nucleus of the stria terminalis in the cardiovascular response to acute restraint stress in rats. Stress, 12: 268 – 278.

[8] Debnath, J.; Prakash, T.; Karaki, R.; Kotresha, D. and Sharma, P. (2011): An experimental evaluation of anti-stress effects of Terminaliachebula. J. Physiol. Biomed. Sci., 24: 13-19.

[9] El-Desouki, N. I.; Afifi, D.F; El-Refaiy, A.I and Talaat, H. (2014): Age- Related Changes in Histological and Cytoskeletal Intermediate Filaments of Mammalian Thyroid Glands and the Prophylactic Role of Vitamin E. Global Veterinaria, 13 (4): 511-519.

[10] El-Desouki, N. I.; El-Refaiy, A. I.; Afifi, D. F. and Abdel-Kader, A. A. (2012): Histological, histochemical and immunohistochemical studies on the cardiac muscle of albino rat under immobilization stress and the possible curative role of diazepam. Egypt. J. Exp. Biol. (Zool.), 8(2): 273 - 285.

[11] El-Desouki, N. I.; El-Refaiy, A. I.; Gabry, M. S., Ibrahim, M. A. and Mohamed, H. N. (2013): Effect of immobilization stress on the cytoskeletal intermediate filaments of rat stomach and the possible curative role of diazepam. J. Life Sci., 10 (2): 2211 – 2219.

[12] El-Desouki, N. I.; Gabry, M. S. and Nagi, H. M. (2015a): Beneficial role of diazepam in the histological alterations of colon post immobilization stress-induced in adult albino rats. IJSER, 6 (5): 432-440.

[13] El-Desouki, N. I.; Gabry, M. S. and Nagi, H. M. (2015b): The cytoskeletal intermediate filaments alterations of rat colon after exposure to immobilization stress and the curative role of diazepam. IJSER, 6 (7): 445-459.

[14] Engel, J.; Solomon, M. and Jean, A. (2007): Epilepsy A comprehensive Textbook. 2nd ed.Vol. 2. Philidilphia, P A: Lippincott Williams and Wilkins, 1433-1466.

[15] Flitney, E. W.; Kuczmarski, E. R.; Adam, S. A. and Goldman, R. D. (2009): Insights into the mechanical properties of epithelial cells: the effects of shear stress on the assembly and remodeling of keratin intermediate filaments. FASEB J., 23(7):2110-2119.

[16] Gabry, M. S.; El-Desouki, N. I.; El-Refaiy, A. I.; Ibrahim, M. A. and Mohamed, H. N. (2011): Histological study on the stomach of immobilized-stressed albino rat and the curative role of diazepam. Egypt. J. Exp. Biol. (Zool.), 7(2): 153-161.

[17] Garabadu, D. and Krishnamurthy, S. (2014): Diazepam potentiates the antidiabetic, antistress and anxiolytic activities of metformin in Type-2 Diabetes mellitus with cooccuring stress in experimental animals. Inter. Biomed. Res., 2014: 1-15.

[18] Gligorijević, J.; Djordjević, B.; Petrović, A.; Radirević A. and Stojanović, S. (2010): Expression of CD34 in cirrhotic liver-reliance to dedifferentiation. Vojnosanit Pregl., 67(6):459-462.

[19] Gold, M.; Blanchet, M. R.; Samayawardhena, L. A.; Bennett, J.; Maltby, S.; Pallen, C. J. and McNagny, K. (2010): CD34 function in intracellular signaling and mucosal inflammatory disease development. Allergy Asthma & Clinical immunology, 6(3): P15

[20] Hassan, N. M., Harbi, S. O., Shomo, A. I., El-Tahir , Y. I. and Ahmed, M. E. (2014): Cytokeratin 18 and vimentin expression in breast cancer tissue from Sudanese patients. Ann. Biol. Res., 5(1): 9 – 16.

[21] Karantza, V. (2011): Keratins in health and cancer: more than epithelial cell markers. Oncogene, 30: 127-138.

[22] Korita, P. V.; Wakai, T.; Ajioka, Y.; Inouel, M.; Takamura, M.; Shirai Y. and Hatakeyama, K. (2010): Aberrant expression of vimentin correlates with dedifferentiation and poor prognosis in patients with intrahepatic cholangio-carcinoma. Anticancer Res., 30: 2279-2286.

[23] Ku, N. O.; Soetikno, R. M. and Omary, M. B. (2003): Keratin mutation in transgenic mice predisposes to Fas but not TNF-induced apoptosis and massive liver injury. J. Hepatol., 37: 1006-1014.

[24] Ku, N. O.; Strnad, P.; Zhong, B. H.; Tao, G. Z. and Omary, M. B. (2007): Keratins let liver live: Mutations predispose to liver disease and cross linking generates Mallory-Denk bodies. J. Hepatol., 46:1639-1649.

[25] Ku, N. O., Toivola, D. M.; Strand, P and Omary, M. B. (2010): Cytoskeletal keratin glycosylation protects epithelial tissue from injury. Nature cell Biology, 12(9): 876-894.

[26] Kulkarni, M. P. and Juvekar, A. R. (2008): Attenuation of acute and chronic restraint stress-induced perturbations in experimental animals by Nelumbo nucifera Gaertn. Ind. J. Pharm. Sci., 70(30): 327-332.

[27] Kumar, A.; Goyal, R. and Prakash, A. (2009): Possible GABAergic mechanisms in the protective effect of allopregneonolone against immobilization stress. Eur. J. Pharmacol., 602: 343-347.

[28] Kumar, N.;, Robidoux, J.; Daniel, K. W.; Guzman, G.; Floering, L. M. and Collins, S. (2007): Requirement of vimentin filament assembly for beta3-adrenergic receptor activation of ERK MAP kinase and lipolysis. J. Biol. Chem., 282:9244-9250.

[29] Liem, R. K.H. and Messing, A. (2009): Dysfunctions of neural and glial intermediate filaments in disease. J. Clin. Invest., 119: 1814-1824.

[30] Loufrani, L. and Henrion, D. (2008): Role of the cytoskeleton in flow (shear stress)-induced dilation and remodeling in resistance arteries. Med. Biol. Eng. Comput., 46(5): 451-460.

[31] Lttiyavirah, S. P. And Sajid, K. P. (2014): Behavioral assessement of Mikania micrantha Kunth roots in Wistar albino rats. J. Med. Plants Res., 8 (11): 448 – 453.

[32] Mackie, A. R. and Losordo, D. W. (2011): CD34-positive stem cells in the ttreatment of heart and vascular disease in human beings. Tex. Heart Inst. J., 38(5): 474-485.

[33] Narita, M.; Oussoultzoglou, E.; Chenard, M. P.; Fuchshuber, P.; Rather, M.; Rosso, E.; Addeo, P.; Jaeck, D. and Bachellier, P. (2012): Liver injury due to chemotherapy-induced sinusoidal obstruction syndrome is associated with sinusoidal capillarization. Ann. Surg. Oncol., 19: 2230-2237.

[34] Nieminen, M.; Henttinen, T.; Merinen, M.; Marttila-Ichihara, F.; Eriksson, J. E. and Jalkanen, S. (2006): Vimentin function in lymphocyte adhesion and transcellular migration. Nat. Cell Biol., 8:156-162.

[35] Omary, M. B.; Ku, N. O. and Toivola, D. M. (2002): Keratins: Guardians of the liver. J. Hepatol., 35: 251 -257.

[36] Omary, M. B.; Ku, N.O.; Strnad,P. and Hanada, S. (2009): Toward unraveling the complexity of simple epithelial keratins in human disease. J.Clin. Invest., 119:1794-1805.

[37] Paget,G. E. and Barens, J. M. (1964): Evaluation of drug activities, 2nd ed. Laurence and Bacharach, Academic press, New York.

[38] Pallari, H. M. (2010): Dynamic interplay between the intermediate filament protein nestin and cyclin-dependent kinase 5. PHD. Thesis, University of Turku, Finland.

[39] Pekney, M. and Lane, E. B. (2007): Intermediate filaments and stress. Exp. Cell Res., 313(10): 2244-2254.

[40] Perez-Nievas, B. G.; Hummer Schmidt, T.; Kummer, M. P.; Terwel, D.; Leza, J.C. and Henka, M. T. (2011): Restraint stress increases neuroinflammation independently of amyloid β Levels in amyloid precursor protein/PS1transgenic mice. J. Neurochem.,116: 43-52.

[41] Phua, D. C.; Humbert, P. O. and Hunziker, W. (2009): Vimentin regulates scribble activity by protecting it from proteasomal degradation. Mol. Biol. Cell 20:2841-55.

[42] Pusztaszeri, M. P.; Seelentag, W. and Bosman, F.T. (2006): Immunohistochemical expression of endothelial markers CD31, CD34, von-Willebrand factor and Fli-1 in normal human tissues. J. Histochem. Cytochem., 54: 385 - 395.

[43] Rahal, A.; Mehra, D.; Rajesh, S.; Sing, V. and Ahmad, A. A. (2009): Prophylactic efficacy of Podophyllum hexandrum in alleviation of immobilization stress-induced oxidative damage in rat. J. Nat. Prod., 2: 110-115

[44] Sanchez, O.; Arnau, A.; Pareja, M.; Poch, E.; Ramirez, I. and Soley, M. (2002): Acute stress-induced tissue injury in mice: differences between emotional stress and social stress. J. Cell stress & Chaperones, 7(1): 36-46.

[45] Sanchez, O.; Viladrich, M.; Ramirez, I. and Soley, M. (2007): Liver injury after an aggressive encounter in male mice. Am. J. Physiol. Regul. Inter. Comp. Physiol., 293: 1908-1916.

[46] Satelli, A. and Li, S. (2011): Vimentin as a potential molecular target in cancer therapy or vimentin, an overview and its potential as a molecular target for cancer therapy. Cell Mol. Life Sci., 68(18): 3033–3046.

[47] Scherberich, A.; Di Maggio, N. and McNagny, K. M. (2013): A familiar stranger: CD34 expression and putative functions in SVF cells of adipose tissue. World J. Stem Cells, 5(1): 1-8

[48] Soliman, A. A. (2006): Effect of stress on the mammary gland of lactating albino rat: A light and electronic microscopic study. Egypt. J. Hisotol., 29(2): 259-268.

[49] Strnad, P.; Harada, M.; Siegel, M.; Terkeltaub, R.A.; Graham, R. M. and Khosla, C. (2007): Transglutaminase 2 regulates Mallory body inclusion formation and injuryassociated liver enlargement. Gastroenterology, 132: 1515-1526.

[50] Toivola, D. M.; Nieminen, M. I.; Hesse, M.; He, T.; Baribanlt, H.; Magrin, T. M.; Omary, M. B. and Eriksson, J. E. (2001): Disturbances in hepatic cell-cycle regulation in mice with assembly-deficient keratins 8/18.J. Hepatol., 34: 1174-1183.

[51] Toti, P.; Regoli, M.; Nesi, G.; Occhini, R.; Bartolommei, S.; Fonzi.; L. and Bertelli, E. (2005): Nestin expression in normal adrenal gland and adrenocortical tumors. Histol. Histopathol., 20: 115-120.

[52] Tsuruta, D. and Jones, J. C. (2003): The vimentin cytoskeleton regulates focal contact size and adhesion of endothelial cells subjected to shear stress. J. Cell Sci., 116(24):4977-4984

[53] Ulrich-Lai, Y. M.; Figueiredo, H. F.; Ostrander, M. M.; Choi, D. C.; Engeland, W. C. and Herman, J. P. (2006): Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am. J. Physiol. Endocrinol. Metab., 291(5): 965-973.

[54] Vij, M.; Patra, S. and Rela M. (2012): Pigmented hepatocellular adenoma with complete CD34 immunostaining pattern: A diagnostic dilemma. Indian J. Pathol. Microbiol., 55: 528 - 530.

[55] Zhao, Y.; Wang, Z.; Dai, J.; Chen, L.; Huang, Y. and Zhan, Z. (2012): Benificial effects of benzodiazepine diazepam on chronic stress-induced impairment of hippocampal structural plasticity and depression-like behavior in mice. Behav. Brain Res., 228: 339-350.