Original Research Article

Effect of sleep deprivation on the liver, kidney and heart: histological and immunohistochemical study

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ABSTRACT

Background: Sleep deprivation is verging on a public health epidemic. It induced mild organ injuries through oxidative stress. The present study aimed at evaluating possible histopathological and immunohistochemical alterations caused by sleep deprivation on the liver, heart and kidney.
Methods: 36 Wistar senile male albino rats were divided into two groups; control and experimental. Experimental rats were placed in a plastic cage, with grid floor placed inside it. The cage was filled with water to 1 cm below the grid surface. The stainless-steel rods of the grid were set 2 cm apart from each other. Loss of muscle tone associated with sleep caused them to touch the water and wake up. At the end of the experiment, animals were sacrificed by decapitation on days 1, 3 and 5. Liver, kidney and heart were obtained and dissected.
Results: In SSD1 group, liver cells showed early stage of hepatic steatosis while mucolipidosis within hepatocytes was detected in SSD3 and the degree of steatosis increased in SSD5. Renal glomerular congestion was detected in SSD1. In SSD3 vascular congestion of glomerular tufts and apoptosis of the lining tubular epithelium was seen. Renal casts were formed in SSD5. In SSD1 lysis of myocardial fibrils was seen. Myocardial fibers degeneration represented by increase sarcoplasmic eosinophilia in SSD3. Sarcoplasmic vacuolation and nuclear chromatolysis increased in SSD5. Staining with GFAP and P53 showed various results among the liver, heart and kidney.
Conclusions: Sleep deprivation might induce multiple organ injury through oxidative stress causing altered histological appearance.

Keywords: Sleep deprivation, Oxidative stress, Melatonin, GFAP, P53

INTRODUCTION

Sleep is a restorative process that plays a major role in the balance of psychological and physical health.[1] Sleep is beneficial for every organ in the body, and sleep deprivation leads to disturbances that cause irreparable damage.[2] Reduction in sleep duration and sleep quality is progressively common in modern society and is likely linked to changes in the socio-economic environment and lifestyle.[3] The percentage of adults reported sleeping 6 hours or less increased by 5% - 6% between 1985 and 2004 and with the advancement of age, sleeping hours progressively decrease.[4]

Clinical evidences suggest that both short and long habitual sleep loss are associated with an increased risk of mortality, hypertension, atherosclerosis and insulin resistance, as well as the stress-induced remodeling of the brain regions that participate in the regulation of memory, executive function and anxiety.[6-10] Short sleep duration is associated with self-rated poor health and elevated body mass index (BMI).[11,12] Sleep is a vital regulator of cardiovascular function, both in the physiological state and in disease conditions.[13] Sleep disorders are related to an increased prevalence of cardiovascular disease and even an independent risk factor for the development of that disease.[14] Moreover, sleep deprivation causes a

decrease in liver glutathione and catalase activity without compensatory increases in other enzymatic anti-oxidants.[15]

In subjects suffering sleep deprivation there is a desynchronization of cellular function, which inevitably leads to metabolic disturbances as sleep deprivation results in uncompensated oxidative stress.[16,17] Oxidative stress is an imbalance between the formation and elimination of reactive oxygen/nitrogen species and is associated with several adverse outcomes such as cancer, immunodeficiency disease, neurological diseases, and cardiovascular diseases.[18] Furthermore, it is involved in the mechanisms of aging, atherosclerosis, diabetes, and neuro-degenerative disorders.[19] Free radicals accumulate during waking because of enhanced metabolic activity and are responsible for the effects of sleep deprivation.[20] By reviewing the literature, few studies were found investigating the histopathological and immuno-histochemical effects of sleep deprivation on the liver, heart and kidney. Thus, the focus of the present study was to evaluate the possible histopathological and immuno-histochemical alterations caused by sleep deprivation on the liver, heart and kidney.

METHODS

Experimental animals

After obtaining the approval of Ethical Committee of Animal Research Ethics (CARE) – Faculty of Medicine – Ain Shams University, Thirty–six healthy Wistar senile male albino rats (weighing 230-290 gm) were obtained and locally bred at the animal house of the Medical Research Center (MRC), Faculty of Medicine, Ain Shams University. Rats were housed in plastic cages, two rats per cage, and were left one week before the start of the experiment to acclimatize to experimental conditions. The rats were exposed to 12 hours light/dark cycle and allowed daily diet and free water access (ad libitum) with suitable environmental conditions and good ventilation.

Experimental design

The rats were divided into two groups (18 rats per group)

Group I: Senile control group (SC) (18 rats).

Rats were housed under normal (pathogen-free) conditions, and were subdivided into three subgroups:

Subgroup (SC1): six rats were sacrificed after one day from the start of the experiment.

Subgroup (SC3): six rats were sacrificed after three days from the start of the experiment.

Subgroup (SC5): six rats were sacrificed after five days from the start of the experiment.

Group II: Senile sleep deprivation group (SSD) (18 rats).

Rats were placed in a plastic cage (30 × 8 × 4 cms), with grid floor (29 × 5 × 7 cms) placed inside the plastic cage. The cage was filled with water to 1 cm below the grid surface. The stainless steel rods of the grid (3 mm wide) were set 2 cm apart from each other. The loss of muscle tone associated with sleep caused them to touch the water and wake up. This model does not impose restriction of movement or social isolation.[21]

Subgroup (SSD1): six rats were sacrificed after one day from the start of the experiment.

Subgroup (SSD3): six rats were sacrificed after three days from the start of the experiment.

Subgroup (SSD5): six rats were sacrificed after five days from the start of the experiment.

At the end of the experiment, the animals were sacrificed by decapitation on days 1, 3 and 5 after start of the experiment. The liver, kidney and heart were immediately obtained and dissected. Disposal of animal remains was done according to the regulations of the animal house.

Processing of samples

Preparation of paraffin blocks

Samples of liver, heart and kidney from the rats were dissected and placed in 10% formalin. The samples were dehydrated using a graded percentage of ethanol and then fixed in paraffin wax for 1 hour to form paraffin blocks. The blocks were trimmed and cut into 4 μm thick sections. Some sections were stained with hematoxylin and eosin (H&E), and then mounted using Depex-Polystyrene dissolved in xylene mountant to examine the histological structure of the liver, kidney and heart in the different groups.

Immunohistochemical staining procedures

Every fifth paraffin section was mounted on polysine-coated slides or on charged slides to be immuno-histochemically stained to show either glial fibrillary acidic protein (GFAP) or p53. All sections were examined and photographed using light microscope (Olympus 268 M microscope).

Sections were stained using the avidin-biotin immunohistochemical technique for detection of glial fibrillary acidic protein (GFAP) within hepatic stellate cells. Universal kits for GFAP manufactured by Biogen Inc. (Cambridge, Massachusetts, USA) were obtained from Dako Company (Egypt). Sections were deparaffinized, rehydrated and rinsed in tap water, embedded in 3% hydrogen peroxide for 10 minutes then immersed in antigen retrieval solution. Nonspecific

protein binding was blocked using blocking solution [phosphate buffer solution (PBS) plus 10% nor- mal goat serum]. Sections were incubated for 2 hours with the diluted primary antibody using PBS at different dilutions (1/500, 1/200 and 1/100) for biotinylated monoclonal mouse antibody for GFAP. Drops of streptavidin peroxidase were added for 20 minutes then washed with PBS for 5 minutes. Diaminobenzidine (DAB) was added to as chromogen and Mayer's hematoxylin was used as a counter stain. For negative control, the primary antibody was replaced by PBS.[22]

Other paraffin sections were put into FSG 120 (FSG120-T/T Controlled Antygen Retrieval, Milestone, Italy) at 110°C after paraffin was removed and hydration performed in citric buffer (pH 6.0). The pressure was adjusted to 2 bar for 10 min to obtain antigen retrieval. After cooling, the samples were placed in 3% hydrogen peroxide solution for 5 min, then washed in distilled water and placed in fresh buffered Tris for 5 min. The histological preparation was covered with the primary anti p53 antibodies in dilution of 1:50 (anti-human p53 protein, DakoCytomation, Denmark) incubated at room temperature for 30 min and placed in a fresh Tris bath for 5 min. Then, the secondary antibody was added

(biotinylated link universal from the commercial kit LSAB: DakoCytomation, Denmark), incubated at room temperature for 30 min before washing in fresh Tris for 5 min. Another secondary antibody was added (streptavidin-HRP from LSAB kit) for 30 min and the section was placed in fresh Tris for 5 min. The preparation was covered with DAB stain (3, 3-diaminobenzidine tetrahydrochloride, DAB Chromogen, DakoCytomation, Denmark) for 10 min and then bathed in distilled water.[23]

RESULTS

Histopathological findings

Group I: SC group

Examination of hepatic, renal, and cardiac sections of the subgroups SC1, SC3, and SC5 showed almost similar integrity of the tissues and cells. Examination of the hepatic sections, showed normal hepatocytes arranged in cords (Figure 1A). Examination of the renal sections, showed normal renal glomeruli and tubules (Figure 1B). Examination of the cardiac sections, showed normal myocardial fibers (Figure 1C).
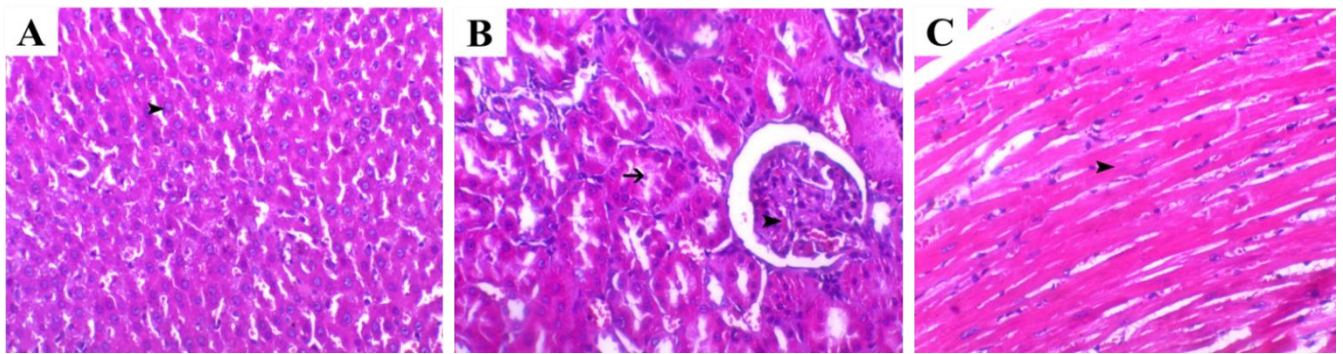

Figure 1: A photomicrograph of a section of a rat's liver (A) showing normal hepatocytes arranged in cords (arrowhead), a section of a rat's kidney; (B) showing normal renal glomeruli and tubules (arrowhead and arrow respectively), a section of a rat's heart; (C) showing normal myocardial fibers (arrowhead) (group I SC) (H&E, X200).
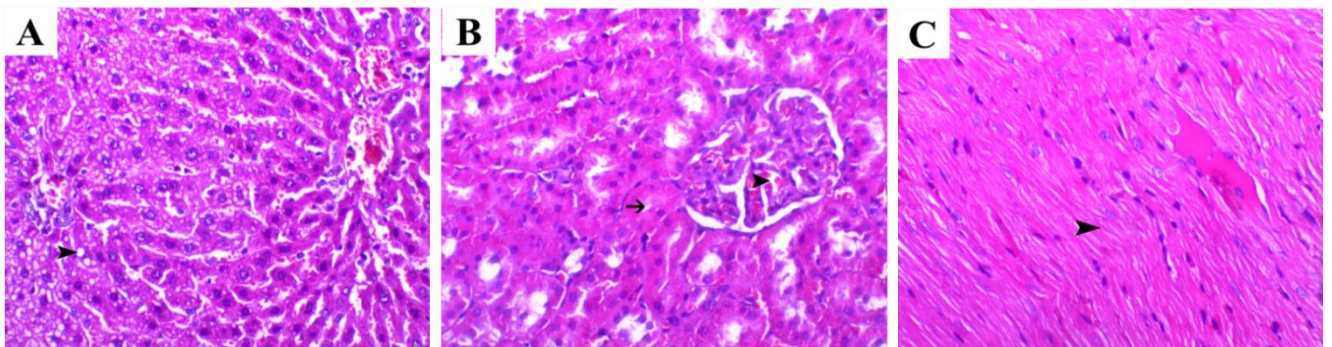

Figure 2: A photomicrograph of a section of a rat's liver (A) showing early stage of hepatic steatosis (arrowhead), a section of a rat's kidney; (B) showing renal glomerular congestion (arrow) and tubular epithelium swelling (arrowhead), a section of a rat's heart; (C) showing lysis of myocardial fibrils (arrowhead) (subgroup SSD1) (H&E, X200).
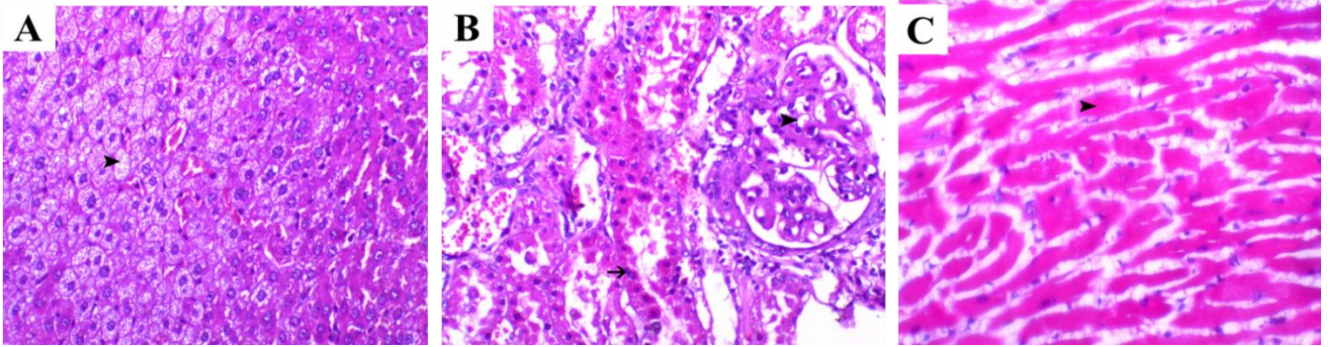
Figure 3: A photomicrograph of a section of a rat's liver (A) showing mucolipidosis within hepatocytes (arrowhead), a section of a rat's kidney; (B) showing vascular congestion of glomerular tufts (arrowhead) and apoptosis of the lining tubular epithelium (arrow), a section of a rat's heart; (C) showing myocardial fibers degeneration representing by increase sarcoplasmic eosinophilia (arrowhead) (subgroup SSD3) (H&E, X200).
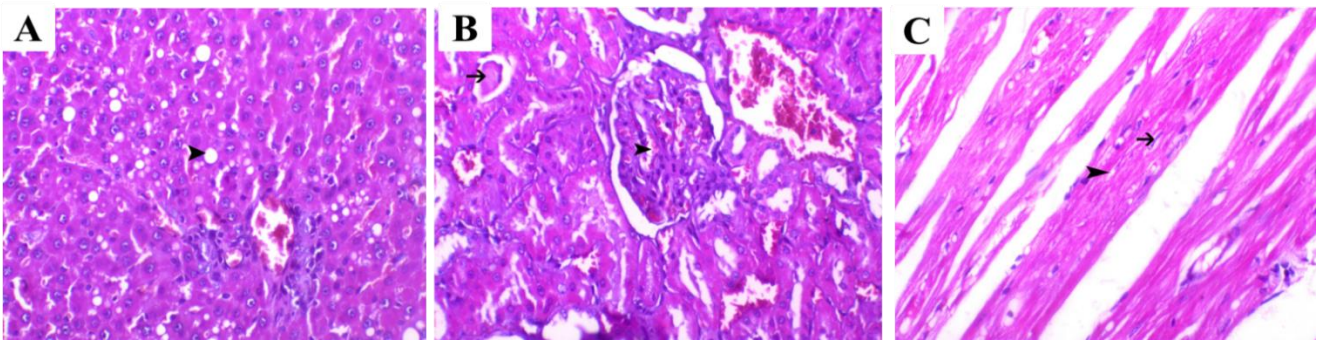

Figure 4: A photomicrograph of a section of a rat's liver (A) showing increase the degree of steatosis (arrowhead), a section of a rat's kidney; (B) showing renal glomerular tufts congestion and (arrowhead) and renal casts (arrow), a section of a rat's heart; (C) showing sarcoplasmic vacuolation (arrowhead) and nuclear chromatolysis (arrow) (subgroup SSD5) (H&E, X200).
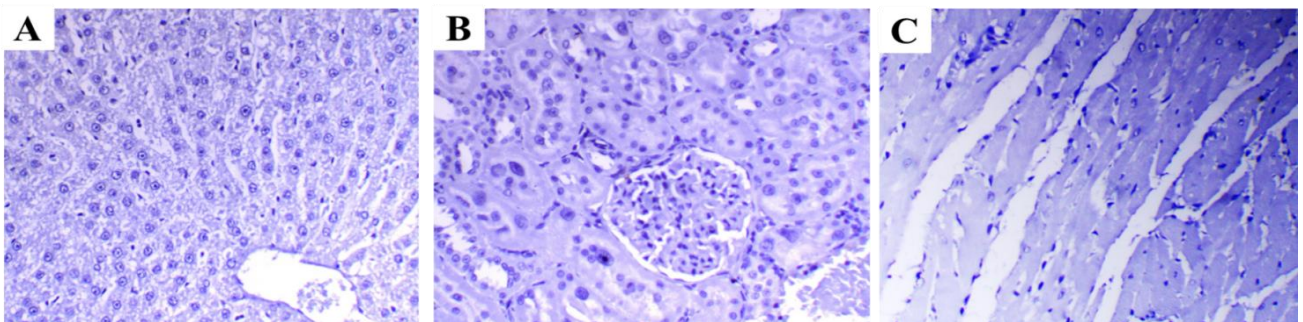

Figure 5: A photomicrograph of a section of a rat's liver (A) showing negative GFAP immunohistochemical staining, a section of a rat's kidney; (B) showing negative GFAP immunohistochemical staining, a section of a rat's heart; (C) showing negative GFAP immunohistochemical staining (group I SC) (GFAP X200).

Group II: SSD group

In subgroup (SSD1)

Examination of the hepatic sections, showed early stage of hepatic steatosis (Figure 2A). Examination of the renal sections, showed Renal glomerular congestion and tubular epithelium swelling (Figure 2B). Examination of the cardiac sections, showed lysis of myocardial fibrils (Figure 2C).

In subgroup (SSD3)

Examination of the hepatic sections, showed mucolipidosis within hepatocytes (Figure 3A). Examination of the renal sections, showed vascular

congestion of glomerular tufts and apoptosis of the lining tubular epithelium (Figure 3B). Examination of the cardiac sections, showed myocardial fibers degeneration represented by increase sarcoplasmic eosinophilia (Figure 3C).

In subgroup (SSD5)

Examination of the hepatic sections, showed an increase in the degree of hepatic steatosis (Figure 4A). Examination of the renal sections, showed renal glomerular tufts congestion and renal casts formation (Figure 4B). Examination of the cardiac sections, showed

sarcoplasmic vacuolation and nuclear chromatolysis (Figure 4C).

Immunohistological results

Immuno-stained sections for glial fibrillary acidic protein (GFAP)

Group I: SC group

Examination of the hepatic, renal and cardiac sections of the subgroups SC1, SC3, and SC5 revealed negative GFAP immunohistochemical staining (Figures. 5 A-C).
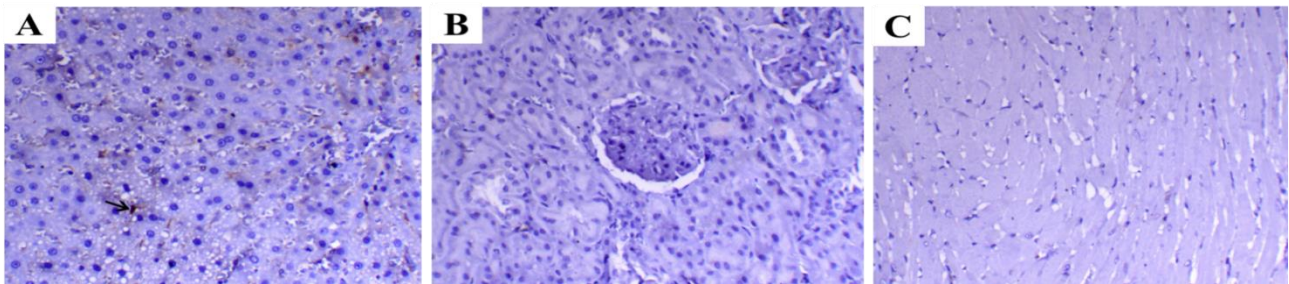

Figure 6: A photomicrograph of a section of a rat's liver (A) showing immunoexpression of GFAP within the sinusoidal Kuffer's cells (arrow), a section of a rat's kidney; (B) showing negative GFAP immunohistochemical staining, a section of a rat's heart; (C) showing negative GFAP immunohistochemical staining (subgroup SSD1) (GFAP X200).
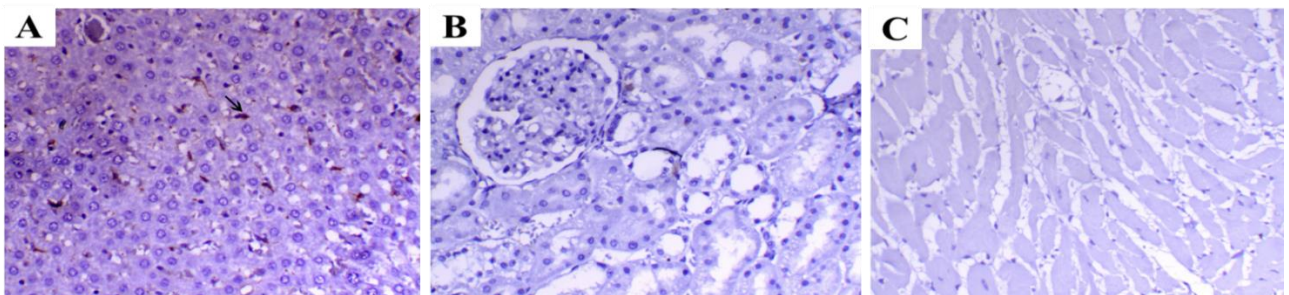

Figure 7: A photomicrograph of a section of a rat's liver (A) showing increased immunoexpression of GFAP within the sinusoidal Kuffer's cells (arrow), a section of a rat's kidney; (B) showing negative GFAP immunohistochemical staining, a section of a rat's heart; (C) showing negative GFAP immunohistochemical staining (subgroup SSD3) (GFAP X200).
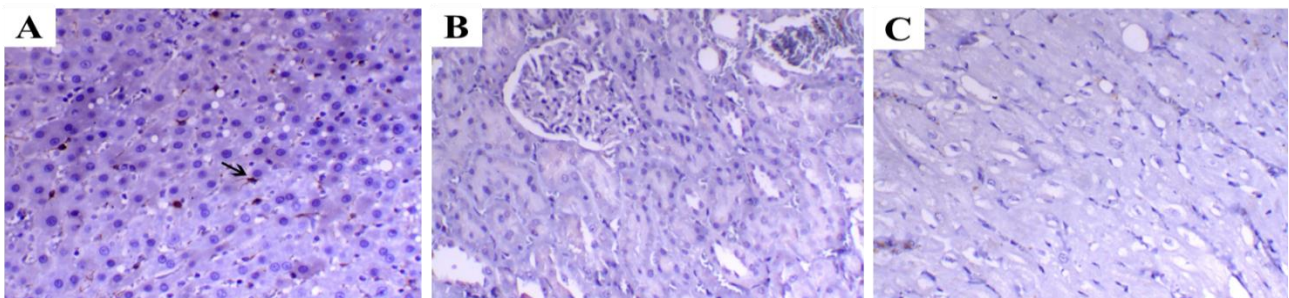

Figure 8: A photomicrograph of a section of a rat's liver (A) showing increased immunoexpression of GFAP within the sinusoidal Kuffer's cells associated with increase their processes (arrow), a section of a rat's kidney; (B) showing negative GFAP immunohistochemical staining, a section of a rat's heart; (C) showing negative GFAP immunohistochemical staining (subgroup SSD5) (GFAP X200).

Group II: SSD group

In subgroup (SSD1)

Examination of the hepatic sections showed positive GFAP immunohistochemical staining within the sinusoidal Kupffer cells (Figure 6A). Examination of both renal and cardiac sections showed negative GFAP immunohistochemical staining within the sinusoidal Kupffer cells (Figures 6 B and C).

In subgroup (SSD3)

Examination of the hepatic sections showed increase expression of GFAP immunohistochemical staining within the sinusoidal Kupffer cells (Figure 7A). Examination of both renal and cardiac sections showed negative GFAP immunohistochemical staining within the sinusoidal Kupffer cells (Figures 7 B and C).

In subgroup (SSD5)

Examination of the hepatic sections showed increase expression of GFAP immunohistochemical staining within the sinusoidal Kupffer cells associated with increase in their processes (Figure 8A). Examination of

both renal and cardiac sections showed negative GFAP immunohistochemical staining within the sinusoidal Kupffer cells (Figures 8 B and C).

Immuno-stained sections for P53

Group I: SC group

Examination of the hepatic sections showed scarcely positive P53 immunohistochemical staining within the hepatocytes (Figure 9A). Examination of the renal sections showed mild positive P53 immunohistochemical staining (Figure 9B). Examination of the cardiac sections showed mild positive P53 immunohistochemical staining within the myofibrils (Figure 9C).

Group II: SSD group

In subgroup (SSD1)

Examination of the hepatic sections showed increased expression of P53 within the hepatocytes (Figure 10A). Examination of the renal sections showed mild expression of P53 (Fig. 10B). Examination of the cardiac sections showed increase in the sarcoplasmic expression of P53 (Fig. 10C).
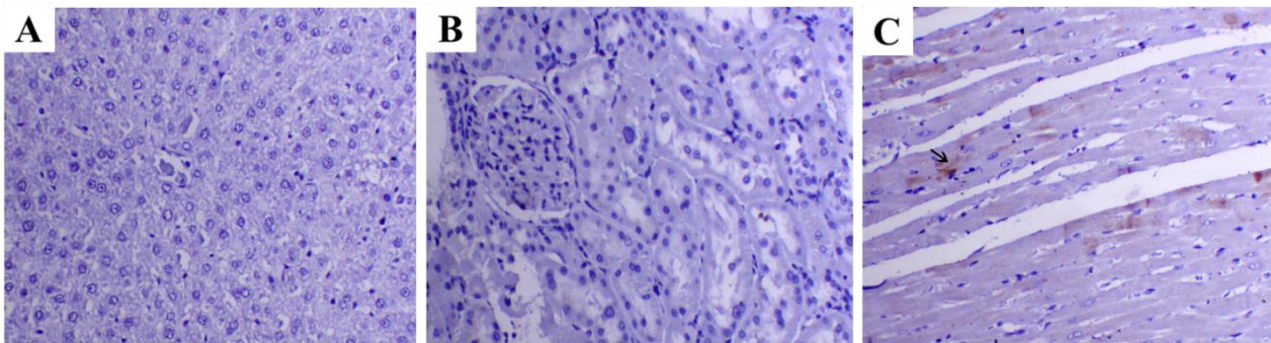

Figure 9: A photomicrograph of a section of a rat's liver (A) showing mild P53 immunohistochemical staining, a section of a rat's kidney (B) showing mild P53 immunohistochemical staining, a section of a rat's heart (C) showing mild sarcoplasmic P53 immunohistochemical staining (arrow) (group I SC) (P53 X200).
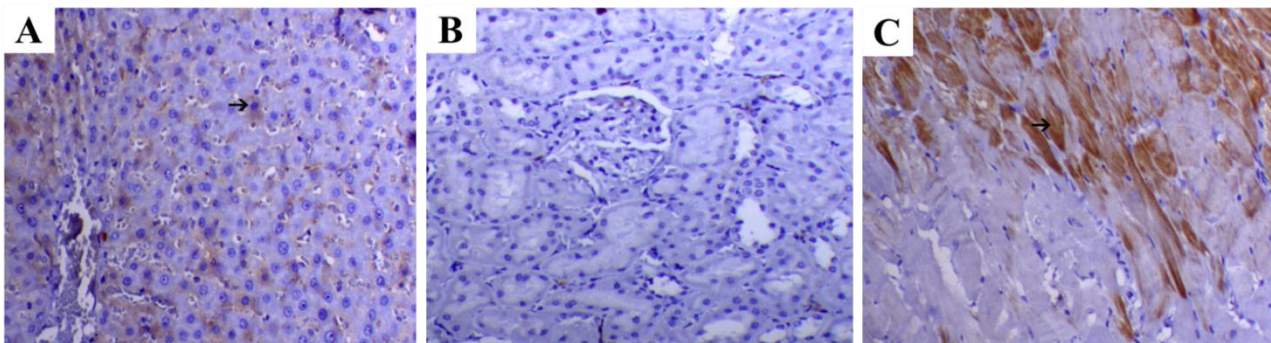

Figure 10: A photomicrograph of a section of a rat's liver (A) showing increase P53 immunohistochemical staining within hepatocytes (arrow), a section of a rat's kidney (B) showing mild P53 immunohistochemical staining, a section of a rat's heart (C) showing increased sarcoplasmic P53 immunohistochemical staining (arrow) (group SSD1) (P53 X200).
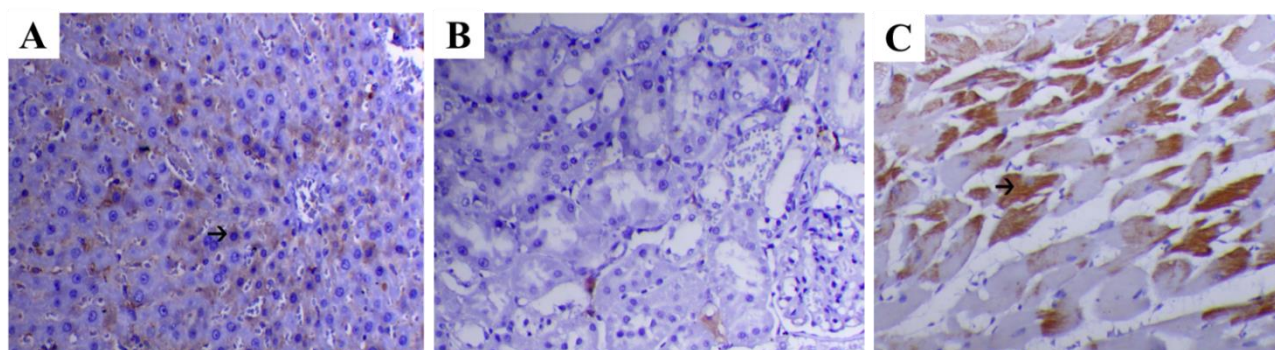
Figure 11: A photomicrograph of a section of a rat's liver (A) showing increase P53 immunohistochemical staining within hepatocytes (arrow), a section of a rat's kidney; (B) showing mild P53 immunohistochemical staining, a section of a rat's heart; (C) showing marked increase in sarcoplasmic P53 immunohistochemical staining (arrow) (group SSD3) (P53 X200)
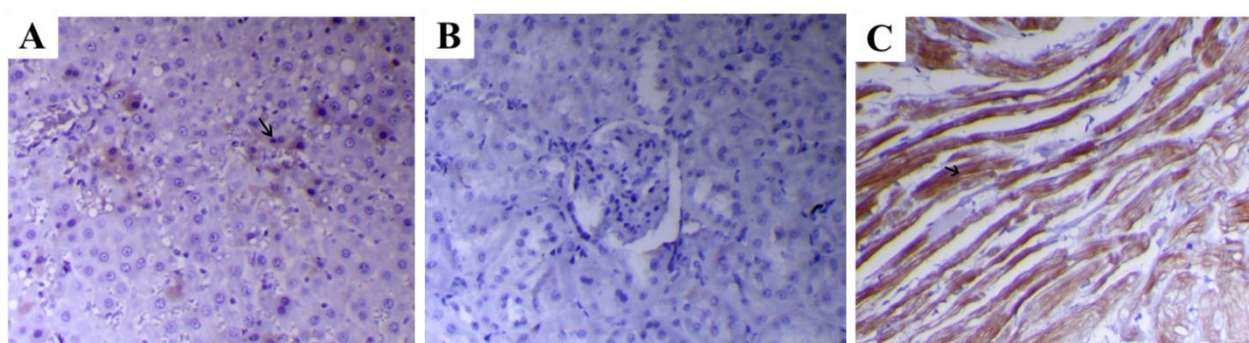

Figure 12: A photomicrograph of a section of a rat's liver (A) increase P53 immunohistochemical staining within hepatocytes (arrow), a section of a rat's kidney; (B) showing mild P53 immunohistochemical staining, a section of a rat's heart; (C) showing marked increase in sarcoplasmic P53 immunohistochemical staining (arrow) (group SSD5) (P53 X200).

In subgroup (SSD3)

Examination of the hepatic sections showed increased expression of P53 within the hepatocytes (Fig. 11A). Examination of the renal sections showed mild expression of P53 (Fig. 11B). Examination of the cardiac sections showed marked increase in the sarcoplasmic expression of P53 (Figure 11C).

In subgroup (SSD5)

Examination of the hepatic sections showed increased expression of P53 within the hepatocytes (Figure 12A). Examination of the renal sections showed mild expression of P53 (Figure 12B). Examination of the cardiac sections showed marked increase in the sarcoplasmic expression of P53 (Figure 12 C).

DISCUSSION

The current outcomes indicate that sleep deprivation-induced moderate multiorgan injury. Liver cells showed early stage of hepatic steatosis which increased by increasing the hours of sleep deprivation indicating liver injury. The liver sections revealed interesting positive reaction with GFAP. The reactions mostly seen within the sinusoidal Kupffer cells, and showed marked increased in their numbers and processes with more deprivation of sleep. The liver showed an increase in the number of positive cells which mostly revealed cytoplasmic P53 expression. Renal injury was in the form of glomerular congestion and vascular congestion of glomerular tufts, tubular epithelium swelling which ended up with apoptosis of the lining tubular epithelium and formation of renal casts. As for the heart, lysis of myocardial fibrils and degeneration occurred along with sarcoplasmic vacuolation and nuclear chromatolysis. It showed diffuse expression of p53 within the sarcoplasms of myocardial fibers.

While few studies were found investigating the histological and immunohistochemical effects of sleep deprivation on different organs, other studies were found studying the effect of sleep deprivation by assessing the serum levels of different chemicals.

It has been reported that endoplasmic reticulum resident proteins are more susceptible to oxidative stress with

aging.[24] Thus, according to Naidoo et al, sleep deprivation would be a greater insult to aged animals than to young animals and would result in greater cellular stress.[25]

In a study by Periasamy et al, sleep deprivation was found to affect the liver through oxidative stress and inflammation in mice. Sleep deprivation increased serum GOT, GPT, and TBIL indicating liver injury.[26] Liver cytokines were altered in sleep deprived mice. In addition, sleep deprivation increased nitrite level. According to Gopalakrishnan et al, sleep deprivation induced some morphological changes in the liver.[27] Mild necrotic hepatocytes were observed around both central and portal veins. In addition, cytoplasmic enlargement with increased nuclear density and mild swelling or ballooning of hepatocytes was found in sleep deprived animals. It is thought that antioxidant imbalance mediates these alterations. Elevated oxidative stress and insufficient antioxidant activities may result in liver cell injury.[2,15]

Research has shown that there is an obvious circadian rhythm in the blood levels of melatonin released from pineal gland in vertebrates, with levels being higher at night and lower during the day. After synthesis, melatonin is released into the cerebrospinal fluid and blood immediately and then is distributed throughout the body in the systemic circulation.[28] Melatonin protects against free radicals and oxidative stress.[29] Melatonin treatment abolished oxidative stress in the liver of aged rats by preventing of the decreased activity of CAT and the downregulation of Cu,Zn-SOD and GPx gene expression.[30] It can augment the activity of other antioxidants and protect antioxidant enzymes from oxidative damage.[31,32] Melatonin acts on the mitochondrial respiratory chain decreasing electron leak and lowers the generation of free radicals.[33] A high-fat diet leads to oxidative stress with extensive liver steatosis in rats. Melatonin reduced hepatic steatosis and inflammation by lowering serum AST, ALT, liver total cholesterol, and triglycerides in high-fat diet fed rats. So with sleep deprivation, disturbance in the levels of melatonin in blood would affect its functions and aggravate oxidative stress and hence cause hepatic steatosis.[28]

Regarding the effect of sleep deprivation on the heart, a study by Periasamy et al showed an increase in serum CPK, CKMB, and LDH demonstrating myocardial injury.[26] Myocardial TNF-α, IL-1β, and IL-6 were increased in initial 24 h and subsequently decreased in 72 h of sleep deprivation. Myocardial oxidative stress indicated by decreased MDA and NO upon sleep deprivation.

Sleep deprivation has been demonstrated by increased proinflammatory cytokines, blood pressure as well as cortisol levels.[34] It also leads to circadian rhythms disruption that has enormous implications in the pathogenesis of cardiac disease.[35] Circadian rhythms play a vital role in the regulation of cardiovascular physiology. Disruption of diurnal rhythms increases mortality in cardiomyopathic hamsters and exacerbates pressure overload myocardial hypertrophy.[35,36] It affects the levels of melatonin which protect against ischemia-reperfusion injury after myocardial infarction.[37] Diurnal cycling plays a key role in organ growth and renewal and disruption is a key contributor to disease.[35]

Sleep deprivation induced renal dysfunction indicated by elevated BUN.[26] It also leads to circadian rhythms disruption that Integrity of peripheral organs such as the kidney depends on the circadian coordination. Long-term disruption of circadian rhythms, in shift workers, transoceanic flight attendants, or patients with sleep disturbances, has enormous implications in the pathogenesis of renal disease. Circadian clocks provide temporal organization for the proliferation of renal tubular epithelial cells may give evidences about cortical cell apoptosis, and renal pathology.[35]

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