Research Article

Role of Melatonin in Management of Partially Hepatectomized and/or Propagated- Cirrhotic Livers of Rats

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ABSTRACT

The present work aims to evaluate the effect of melatonin on partial hepatectomized rats, propagated- cirrhotic and cirrhotic - partial hepatectomized rats. Hepatic resection was carried out in rats by removing 33 % of liver, while cirrhosis was initiated in liver through intraperitoneal injection of dimethyl nitrosamine (DMN) 200mg/kg body weight twice a week for one month. The effect of melatonin on these experimental groups was achieved through evaluating certain biochemical parameters including liver marker enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin. Also, total protein content and albumin were studied. Moreover, liver antioxidants: glutathione (GSH), catalase, nitric oxide (NO) and lipid peroxide were determined in this study. Adenylate energy charge (AEC), ATP, ADP, AMP, inorganic phosphate and phosphate potential were taken also into consideration. Proinflammatory markers; interleukin -1 (IL-1) and tumor necrosis factor- α (TNF- α) as well as growth factors such as hepatocyte growth factor (HGF) and transforming growth factor- β (TGF- β), were also examined. The biochemical results were documented by histopathological examination of different experimental groups. The present results, clearly demonstrate disturbances in all biochemical parameters ascertained with histopathological observation of liver which reveals vascular congestion, inflammatory changes with congested sinusoids, nuclear changes (pyknosis), and centrilobular necrosis, fatty changes, vascular congestion and fatty changes in centrilobular necrotic liver. Improvement in all biochemical parameters studied was noticed in different therapeutic groups, as a result of treatment with melatonin. These results were run in parallel with the finding of amelioration signs in rat's hepatic architecture. Thus it could be concluded that, melatonin can up-regulate and counteract the inflammatory process, minimize damage of the liver, delay disease progression and reduce its complications.

Keywords: Melatonin, hepatectomized rats, inflammation, cirrhosis, fibrosis.

INTRODUCTION

Under normal conditions, hepatocytes are quiescent cells with a long life span. After partial hepatectomy occurs, however, hepatocytes re-enter the cell cycle accompanied with various growth responses[1]. Regarding the transition from quiescent to proliferative hepatocytes, Seki et al.[1], reported that cytokine signaling after partial hepatectomy (PH). Tumor necrosis factor–alpha (TNF-α) binding to its receptor on Kupffer cells, and myeloid differentiation factor 88 (MyD88), also activates nuclear factor kappa beta (NF-κβ), Interleukin-6 is subsequently released and binds to its receptors gp80 and gp130, which leads to hepatocyte proliferation *via* the activation of the Janus kinase (JAK)/signal transducer, activator of transcription (STAT) and mitogen-activated protein kinase (MAPK) pathnways[2]. After these cytokines have triggered the G0 to G1 transition, cell cycle progression is then regulated by several growth factors. Among the growth factors that regulate liver regeneration, hepatocyte growth factor (HGF) which is a potent angiogenic factor that stimulates endothelial cell motility and proliferation, epidermal growth factor (EGF), and transforming growth factor–alpha (TGF-α), are known to stimulate hepatocytes proliferation [3, 4, 5, 6].

In addition, transforming growth factor-beta 1 (TGF-β1) is known to inhibit proliferation and terminate liver regeneration. In addition to these growth factors, the role of non-parenchymal cells during liver regeneration has been a focus of interest. Vascular endothelial growth factor (VEGF) expression increases after PH, and sinusoidal endothelial cell proliferation has been shown to occur during liver regeneration [6].

In the liver of hepatectomized rats, demonstrated a significant reduction of oxidative damage to lipids and proteins together with increased GSH, which is the main intracellular antioxidant, and decreased GSSG.[6] These alterations are indicative of low oxidative stress in the regenerating liver 48 h after partial hepatectomy. Studies on liver oxidative state during the early phase of liver regeneration (up to 24 h after operation) have shown increased oxidative stress, and this could be attributed to the surgical procedure or to a reactive response of the reduced organ to compensate for the extra functional load[7, 8]. It has been recently shown that hepatic lipid peroxidation peaks at 24 h after partial hepatectomy when GSH content is minimum, while both parameters were normalized at 48 h[9]. Investigations on oxidative stress beyond the early phase of liver regeneration have shown a significant decrease of lipid peroxidation at 24, 48, and 72 h after hepatectomy[10, 11, 12]. This effect has been attributed to increase levels of lipid soluble antioxidants like microsomal alpha tocopherol at times of maximal DNA synthesis[11, 12].

A hepatic resection (partial hepatectomy) is invasive for cirrhotic patients because postoperative complications, such as hepatic disturbance sometimes resulting in hepatic failure and pulmonary disturbances, are frequent and serious[13]. In another study, In cirrhotic rats, functional restoration of the liver after 1/3 hepatectomy was advanced in comparison with morphological restoration.[14] Partial hepatectomy seemed to promote functional restoration of the cirrhotic liver [14]. It was also, found that the proliferative rate of hepatectomized-cirrhotic liver was found to be definitely higher than the rate of the non- operated group, but significantly less than that of normal liver [15].

The regeneration of normal and cirrhotic liver has been very well demonstrated after partial hepatectomy; although the tissue regenerated by cirrhotic liver is also cirrhotic. The structural differences of the regenerated tissues between normal and cirrhotic livers may also indicate different regeneration capacities. When the cirrhotic liver regeneration rats was compared with the normal liver regeneration rats, rates of liver regeneration in the cirrhotic rats were significantly depressed and cirrhotic livers revealed a significantly depressed capacity for regeneration following PH[15]. The thoroughly investigated and well-established capacity for regeneration of normal and cirrhotic livers may lead to further encouraging studies on factors that affect regeneration of a healthy liver after cirrhosis. The autocrine, paracrine and endocrine factors thought to influence normal liver regeneration after cirrhosis remains to be determined. Many growth factors and cytokines, most notably hepatocyte growth factor, transforming growth factora, interleukin-6, tumor necrosis factor a, insulin, norepinephrine, gastrin, prostagl and in E2, calcium and vitamin D appear to play important roles in the process of liver regeneration[16, 17, 18].

Oxidative stress may represent a common link between chronic liver damage and hepatic fibrosis[19]**.** Several lines of evidence have suggested the important role of oxidative stress in the etiopathogenesis of hepatic fibrosis. Oxidative stress aggravates liver fibrosis *via* hepatic stellate cell activation and lipid peroxidation stimulates the collagen gene transcription in cell culture of fibroblasts and hepatic stellate cells (HSC) [19].

Furthermore, proinflammatory cytokines including tumor necrosis factor-a (TNF-a) and interleukin-1h (IL-1 h) produced by Kupffer cells also play an important role in the initiation and perpetuation of HSC activation [20]. Three important features must be considered when proposing therapeutic strategies in liver cirrhosis; inflammation, oxidative stress and fibrogenesis[21].

Thus, inhibiting oxidative stress and reducing inflammatory cytokines production were regarded useful antifibrotic strategies in the early stages of hepatic fibrogenesis [22, 23].

Melatonin (MEL) or N-acetyl-5-methoxytryptamine is an indole amine produced by pinealocytes in the pineal gland of higher animals from tryptophan, an amino acid, or serotonin, a neurotransmitter, and cyclically released according to a daily cycle of light and darkness[24, 25]. Furthermore, it participates in many important physiological functions, including anti-inflammatory and antioxidant. It detoxifies a variety of free radicals and reactive oxygen intermediates, including the hydroxyl radical, singlet oxygen, peroxynitrite anion and nitric oxide[26]. In both *in vitro* and *in vivo* experiments, melatonin has been found to protect cells, tissues, and organs against oxidative damage induced by a variety of free-radical-generating agents and processes, such as, the carcinogen safrole, CCl_4, ischemia-reperfusion, amyloid-protein, and ionizing radiation [26]. Melatonin, also has been reported to stimulate the activities of enzymes and increase gene expression that improve the total antioxidative defense capacity of the organism, i.e. SOD, glutathione peroxidase, and glutathione reductase[26, 27].

Moreover Wang et al.[23] found that, melatonin is effective in inhibiting oxidative liver damage and protects against alpha-naphthyl iso-thiocyanate (ANIT)-induced liver injury with cholestasis in rats, and suggested that this protective effect is likely due to its antioxidative properties and above all to its capacity to inhibit liver neutrophil infiltration, a critical factor in the pathogenesis of ANIT induced liver injury. Melatonin also could dose-dependently reduce liver lipid peroxide content in CCl_4 treated rats. This indicated that, melatonin exerts a therapeutic effect on CCl_4-induced acute liver injury in rats, possibly through its antioxidant action [23]. In another study done by Jung et al.[28]**,** proved that melatonin decreased expression of inflammatory mediators including tumor necrosis factor-alpha, interleukin (IL)-1b, IL-6, and inducible nitric oxide synthase in the DMN-induced liver injury in rats and also, it increases antioxidant enzymes.

Furthermore, the protective effects of melatonin against CCl_4-induced hepatic injury in rats was investigated by Ebaid et al.[29] who found that, there was an improvement in rats general hepatic architecture that were treated with melatonin. The authors argue this improvement to melatonin effectively reduce oxidative stress, restore the normal concentrations of antioxidant enzymes, and exhibit antihyperlipidemic activity. In addition, lipid peroxidation, a ROS-mediated mechanism, has been implicated in the pathogenesis of various liver injuries and the subsequent liver fibrogenesis that is observed in experimental animals[29, 30]. In addition Ebaid et al.,[29] declared that, the concentration of MDA and hydroperoxide, as they indices of oxidative stress, were elevated in CCl_4-challenged rats. However, decreased concentrations of these two compounds were found in the liver tissues of those CCl_4-rats that were treated with melatonin suggested that this compound can effectively protect against lipid peroxidation. Melatonin exerts

antioxidant protection in different cell organelles, both *in vitro* and *in vivo*[31].

It was found that, the body has an effective defense mechanism to prevent and neutralize damage induced by free radicals. These enzymes constitute a mutually supportive team of defense against ROS[32]. Melatonin was found to significantly restore the normal concentrations of glutathione (GSH) and catalase activity. The catalase enzyme, exists in all aerobic cells, is a hemeprotein that metabolizes the decomposition of hydrogen peroxide H_2O_2 to form oxygen and water. GSH acts as a non-enzymatic antioxidant that reduces the amount of H_2O_2, hydroperoxides and xenobiotic toxicity[33]. In Ebaid et al.[29] study the significant decrease of hydroperoxide in the blood and hepatic tissues confirmed that, the pre-treatment with melatonin could effectively protect against the hepatic lipid peroxidation induced by CCl_4. The antioxidant activity and antilipidemic effects of melatonin may enhance the modulation of blood pressure and most likely play the most important role in the amelioration of the damage to the target organ[34]. GSH is a crucial determinant for cell survival or death in oxidative stress conditions and, thus, it is critical in reducing the toxic effects of CCl_4. So, the elevated hepatic GSH concentrations in the present results in rats treated with melatonin may be due to an increase in the amount of GSH synthesis and/or regeneration.

Hepatic injury was found to be associated with the up-regulation of TNF-α gene expression observed in the CCl_4 treated rats[35]. Consequently, the overproduction of TNF-α contributed to the manifestation of the systemic inflammatory response and ultimately to the development of organ failure. Therefore, the up-regulation of TNF-α clearly explains the hepatic tissue damage and dysfunction observed in the CCl_4 treated rats.[29] Also, hepatic injury in rats leads to elevations of serum AST, ALT and ALP an increased incidence and severity of histopathological hepatic lesions. It was found that the oxidative stability that is induced by the melatonin may mediate a down-regulation of NF -κB activation, results in the suppression of the inflammatory cascade and the low concentrations of TNF-α. Thus, the hepatic injury markers were significantly retarded in the animals that received melatonin. In fact, melatonin significantly attenuated the increased concentrations of the serum liver enzymes induced by CCl_4 and therefore led to the subsequent restoration of these enzymes to the normal concentrations[29].

Thus, the present work aims to evaluate the effect of melatonin hormone on partial hepatectomized rats, propagated- cirrhotic and cirrhotic -partial hepatectomized rats through measuring certain biomarkers in rat's serum and liver. In addition, histopathological observation of liver in different experimental groups was performed.

MATERIALS AND METHODS

Chemicals

All chemicals used in the present study are of high analytical grade, products of Sigma (USA), DHEA manufactured by Natrol, Inc. Chatsworth (USA) and Melatonin manufactured by Puritan's Pride, INC. Okadale, NY (USA).

Animals

Male Wistar albino rats (100: 120g), were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet.

Ethics

Anesthetic procedures and handling with animals were complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt and performed for being sure that the animals not suffer at any stage of the experiment.

Doses and route of administration

Melatonin (50 mg/kg/day), suspended in distilled H_20 and given orally by gastric intubation daily for two weeks[36].

Experimental design

Partial hepatectomy

Rats were partially hepatectomized according to the method of Higgins and Andersson[37].

Induction of cirrhosis

Liver cirrhosis was initiated by intraperetonial injection of dimethyl nitrosamine at a dose of 1 ml (diluted 1:100 with 0.15 M sterile NaCl) per 100 gm body weight. The injections were given on three consecutive days of each week for a period of 3 weeks [38]

Determination of biochemical parameters

AST and ALT enzyme activities were estimated by the method of Reitman and Frankel[39].

Estimation of serum phosphatase activity (ALP)

Alkaline phosphatase enzyme activity was determined according to the method of Belfield and Goldberg.[40]

Estimation of total Bilirubin

Serum total bilirubin was determined according to the method of Walter and Gerade[41], where the reaction between bilirubin and the diazonium salt of sulphanilic acid produced azobilirubin which shows a maximum absorption at 535 nm in an acid medium.

Estimation of total protein

Total Protein content was estimated according to the method of Bradford [42].

Determination of serum albumin

Albumin level was measured in blood serum according to the method of Doumas et al.[43].

Estimation of glutathione concentration (GSH)

Gutathione (GSH) was assayed in liver homogenate according to the method of Moron et al.[44].

Estimation of catalase enzyme

Catalase enzyme was assayed in liver homogenate by kits of colorimetric assay method.

Estimation of Maloaldehyde (MAD)

Malonaldehyde was assayed in hepatic tissue according to the method of Satoh.[45]

Estimation of nitric oxide (NO)

Nitric oxide was determined in tissue liver homogenate by colorimetric assay method according to the method of Montgomery and Dymock[46].

Enzymatic determination of adenosine nucleotides (ATP, ADP and AMP) and inorganic phosphate (Pi) in tissue extracts of Albino rat liver

Adenosine nucleotides were extracted from liver of albino rats using trichloroacetic acid (7% TCA) according to the method of Wijsman[47].

Determination of ATP with hexokinase and glucose-6-phosphate dehydrogenase

The enzymatic determination of adenosine triphosphate (ATP) by spectrophotometric method according to Lamprecht and Trautschold[48].

The concentration of ATP can be calculated according to Bergmeyer and Bernt[49] as follow: ATP (concentration) = ΔE x 4.82 x F µ moles/g wet tissue where F is the dilution factor.

Determination of ADP and AMP

ADP and AMP in tissue extract of albino rats were determined according to the method of Jaworek[50], where

ADP (µ moles/g. wet tissue) $=\Delta E_{(ADP)}$ x 0.666 x F.

AMP (µ moles/g. wet tissue) = ΔE(AMP) x 0.336 x F.

Calculation of Adenylate Energy Charge (AEC)

Adenylate energy charge has been proposed as a measure of the energy potentially available from the adenylate system for cellular metabolism and can be calculated from the following equation according to Atkinson and Walton[51] and is expressed without dimension. TA is the summation of calculation of total adenylate (ADP+AMP+ATP).

Determination of inorganic phosphates (Pi)

Inorganic phosphates (Pi) were determined in the same extract in which ATP, ADP and AMP were determined. The method used was that of Fiske and Subbarow[52].

Calculation of phosphate potential

Phosphate potential is an alternative index used to indicate the free energy status of the tissues and can be calculated from the concentrations ratio of [ATP], [ADP] and [Pi] according to the method of Van Waarde et al.[53] and is expressed without dimension.

Estimation of serum TNF-α, TGF-B1, HGF and IL-1

Rat TNF-α, TGF-B1, HGF and IL-1 immuno assay were determined by Enzyme-linked Immunosorbent Assay Kit.

Histopathological analysis

Liver tissue sections were fixed in formalin and embedded in paraffin. Hematoxylin and eosin (H & E) staining was performed according to the standard procedure[54].

RESULTS

As compared to normal healthy rats, partially hepatectomized rats showed significant ($p \leq 0.05$) increase in liver function enzymes; AST, ALT, ALP, and total bilirubin since the 1st day post operation exhibited the highest percentage of increase amounting to 36.00 %, for ALP, while third day post operation recorded the highest percentages of increase for AST and ALT as they exhibited 160% and 225%, respectively. Total bilirbin showed the highest elevated level one week post operation, where it recorded 262.10 %, **(Table 1).** In addition, cirrhotic rats showed, significant($p \leq 0.05$), increase in liver function enzyme activities; AST, ALT, ALP and total bilirubin level by 280.00, 308.00, 111.04 and 36.72%, post two weeks of induction respectively, as compared to the normal healthy rats **(Table 1).** Moreover, cirrhotic–partially hepatectomized rats demonstrated significant increase in liver function enzyme activities; AST, ALT, ALP, and total bilirubin level where, the 1st day post surgery showed the highest percentage increase of ALT and ALP enzyme activities reached to 208.33 and 363.72 %, respectively. While AST enzyme activity and total bilirubin level recorded the highest percentages of increase two weeks post operation (160.00 and 93.1 %, respectively), as compared to normal healthy rats **(Table 1)**.

Treatment of partially hepatectomized rats with melatonin showed significant increase in AST enzyme activity (80.00%), post 1st day of melatonin treatment, while it recorded the same lowest percentage after one and two weeks (40.00%), where it still recorded significant increase. Although, ALT enzyme activity returned to its normal level post three days of melatonin treatment followed by significant increase one and two weeks post treatment. It was also noticed that, significant ($p \leq 0.05$) increase in ALP enzyme activity after 1st day of

melatonin treatment with percentage increase 167.79%, while it reached to its lowest percentage post two weeks of treatment (40.32%), in spite of it still recorded significant increase. In addition, total bilirubin level demonstrated, significant increase post one day of melatonin treatment (12.64%), and returned to its normal level post two weeks of treatment, where insignificant change was detected as compared to normal healthy rats **(Table 1).**

Table 1: Effect of melatonin treatment on liver function enzyme activities (AST, ALT and ALP), and total bilirubin level in different therapeutic groups

Time	Groups / Parameters	AST	ALT	ALP	T.B
1 Day	1) Normal	0.05 ± 0.002 ^{l}	0.04 ± 0.001 ^{k}	53.90 ± 15.26 ^{p}	1.74 ± 0.05 ^{j}
	2)PH	0.12 ± 0.003 ^{e}	0.12 ± 0.051 ^{f}	242.69 ± 23.20 ^{c}	2.38 ± 0.26 ^{g}
	3)Cirr-PH	0.12 ± 0.013 ^{de}	0.12 ± 0.003 ^{e}	249.95 ± 37.05 ^{a}	3.64 ± 0.32 ^{d}
	4)Cirr-PH. with tt.	0.10 ± 0.005 ^{g}	0.06 ± 0.001 ^{b}	140.58 ± 55.40 ^{g}	1.54 ± 0.25 ^{l}
	5)PH with tt	0.09 ± 0.001 ^{h}	0.07 ± 0.002 ^{b}	141.11 ± 50.41 ^{g}	1.96 ± 0.12 ^{h}
3 Day	1) Normal	0.05 ± 0.002 ^{l}	0.04 ± 0.001 ^{k}	53.90 ± 15.26 ^{p}	1.74 ± 0.05 ^{j}
	2)PH	0.13 ± 0.032 ^{d}	0.13 ± 0.004 ^{d}	228.28 ± 79.42 ^{e}	3.36 ± 0.65 ^{e}
	3) Cirr-PH	0.11 ± 0.052 ^{f}	0.12 ± 0.006 ^{g}	245.53 ± 99.47 ^{b}	2.66 ± 0.52 ^{f}
	4) Cirr-PH.with tt.	0.08 ± 0.001 ^{j}	0.06 ± 0.003 ^{a}	110.21 ± 95.00 ^{j}	1.40 ± 0.03 ^{m}
	5)PH with tt	0.08 ± 0.003 ^{j}	0.04 ± 0.002 ^{k}	120.48 ± 89.42 ^{h}	1.84 ± 0.02 ^{i}
1 week	1)Normal	0.05 ± 0.002 ^{l}	0.04 ± 0.001 ^{k}	53.90 ± 15.26 ^{p}	1.74 ± 0.05 ^{j}
	2)PH	0.13 ± 0.053 ^{d}	0.13 ± 0.039 ^{d}	207.43 ± 34.06 ^{f}	6.30 ± 2.02 ^{a}
	3) Cirr.-PH	0.13 ± 0.024 ^{a}	0.11 ± 0.003 ^{a}	227.61 ± 20.10 ^{e}	3.36 ± 1.02 ^{e}
	4)Cirr.-PH with tt.	0.09 ± 0.002 ^{h}	0.05 ± 0.004 ^{b}	83.94 ± 12.68 ^{m}	1.12 ± 0.05 ^{o}
	5)PH with tt	0.07 ± 0.001 ^{k}	0.06 ± 0.009 ^{b}	102.68 ± 17.11 ^{k}	1.40 ± 0.12 ^{m}
2 weeks	1)Normal	0.05 ± 0.002 ^{l}	0.04 ± 0.001 ^{k}	53.90 ± 15.26 ^{p}	1.74 ± 0.05 ^{j}
	2)PH	0.13 ± 0.021 ^{d}	0.11 ± 0.012 ^{d}	206.49 ± 30.24 ^{f}	5.04 ± 0.02 ^{b}
	3) Cirr-PH	0.15 ± 0.011 ^{c}	0.11 ± 0.011 ^{c}	235.39 ± 23.34 ^{d}	4.76 ± 0.02 ^{c}
	4)Cirr.-PH with tt.	0.08 ± 0.001 ^{j}	0.05 ± 0.004 ^{b}	72.19 ± 13.57 ^{o}	1.26 ± 0.12 ^{n}
	5)Cirr.	0.16 ± 0.015 ^{b}	0.19 ± 0.032 ^{b}	113.75 ± 14.00 ^{i}	2.38 ± 0.02 ^{g}
	6)Cirr.with tt	0.09 ± 0.011 ^{h}	0.05 ± 0.001 ^{b}	97.50 ± 10.00 ^{l}	1.26 ± 0.02 ^{n}
	7)PH with tt	0.07 ± 0.013 ^{k}	0.05 ± 0.002 ^{b}	75.63 ± 3.13 ^{n}	1.68 ± 0.02 ^{j}

Data are means ± SD of 10 rats in each group; AST, ALT expressed as Unit/mL and ALP expressed as (IU/L) and T.B expressed as mg/dl; Statistical analysis is performed using two way analysis of variance (ANOVA) combined with Co-state computer program and Post hoc (LSD). Unshared letters between groups are significant at p value ≤ 0.05.

PH with tt: Partially hepatectomized rats with treatment; Cirr.with tt: Propagated Cirrhotic rats with treatment; Cirr. - PH. with tt: Propagated Cirrhotic-partially hepatectomized with treatment; PH: Partially hepatectomized rats; Cirr.: Propagated Cirrhotic rats; Cirr.-PH.: Propagated Cirrhotic -partially hepatectomized rats.

By comparing propagated-cirrhotic rats, post two weeks of melatonin treatment with normal healthy one, there was significant (p≤0.05) increase in the AST, ALT and ALP enzyme activities with percentages increase 73.3, 25.00 and 80.89%, respectively. While, total bilirubin level exhibited significant decrease (27.5%), as compared to normal healthy rats. As Compared cirrhotic-partially hepactomized rats treated with melatonin to normal control rats, there was significant (p≤0.05) increase in AST, ALT and ALP enzyme activities at different durations of experiment, whereas ALP showed the lowest percentage increase post two weeks (33.94%), and post three days for AST (50%). While, ALT level showed significant increase with the same lowest percentages of increase after one and two weeks (25.00%). Total bilirubin level showed significant decrease at different durations of treatment with percentages decrease 11.49 and 19.5%**,** respectively post one and three days, 35.6 and 27.5%, respectively post one and two weeks of melatonin treatment as compared to normal healthy rats **(Table 1).** It was obvious that, treatment with melatonin showed significant decrease in the liver activities and total bilirubin level in all treated groups as compared to untreated one.

As compared to normal healthy rats, partially hepatectomized rats showed significant (p≤0.05), decrease in total protein content and albumin level with percentages decrease amounting to 67.00, 61.88, 44.85 and 31.4 %, respectively, for total protein content after one, three days, one and two weeks post operation. While, the percentages decrease reached to 59.78, 57.03, 51.50 and 40.2%, respectively, for albumin level, after the same intervals. Furthermore, cirrhotic rats showed significant decrease in total protein content and albumin level with percentages decrease reached to 67. 36 and 79.11%, respectively, as compared to normal healthy rats**.** In addition, cirrhotic-partially hepatectomized rats

showed significant (p≤0.05), decrease in total protein content and albumin level**,** recorded 77.20, 74.71, 62.25 and 52.27%, respectively for total protein content after one, three days, one and two weeks. While, the percentages of reduction reached to 86.20, 84.70, 66.59 and 61.7%, respectively for albumin level after the same intervals as compared to normal healthy rats. With respect to, treatment of hepatectomized rats with melatonin significant decrease was noticed in total protein content and albumin level after one, three days and one week post melatonin treatment with percentages decrease reached to 57.58, 56.23 and 53.27 %, respectively for total protein content and 69.78, 38.89 and 29.33 %, respectively, for albumin level. While, total protein content and albumin level returned to their normal values after two weeks of treatment, where insignificant change was detected as compared to normal healthy rats. Also, cirrhotic rats after two weeks of melatonin treatment, showed significant (p≤0.05), decrease in total protein content and albumin level with percentages decrease 56.8 and 29.3%, respectively, as compared to normal healthy rats**.** Moreover, cirrhotic-partially hepactomized rats, treated with melatonin showed significant decrease in total protein content as well as in albumin level with different intervals, recorded the lowest percentages of reduction, after two weeks of melatonin treatment where insignificant change was observed, as compared to normal control group **(Table 2).** Obvious enhancement post melatonin treatment was demonstrated in the total protein content and albumin level as compared to untreated groups **(Table 2)**.

Table 2: Effect of melatonin treatment on catalase, glutathione level, albumin and total protein in different therapeutic groups

Time	Groups / parameters	Catalase	Glutathione	Total protein	Albumin
1 Day	1)Normal	164.67 ± 44.5 ^{c}	1733.15 ± 133.34 ^{i}	11.15 ± 1.41 ^{a}	4.50 ± 0.57 ^{a}
	2)PH	87.41 ± 20.4 ^{l}	799.29 ± 150.13 ^{m}	3.68 ± 0.58 ^{ij}	1.81 ± 0.01 ^{ghj}
	3) Cirr.-PH	89.00 ± 30.3 ^{l}	933.44 ± 200.47 ^{l}	2.54 ± 0.63 ^{h}	0.62 ± 0.02 ^{l}
	4) Cirr.-PH with tt.	137.44 ± 40.1 ^{fg}	3734.54 ± 289.43 ^{d}	3.71 ± 0.97 ^{de}	2.11 ± 0.66 ^{f}
	5)PH with tt	132.43 ± 50.5 ^{h}	2589.44 ± 200.77 ^{ef}	4.73 ± 0.95 ^{c}	1.36 ± 0.13 ^{j}
3 Days	1)Normal	164.67 ± 44.5 ^{c}	1733.15 ± 133.34 ^{i}	11.15 ± 1.41 ^{a}	4.50 ± 0.57 ^{a}
	2)PH	91.93 ± 34.6 ^{m}	933.01 ± 120.63 ^{l}	4.25 ± 0.54 ^{j}	1.94 ± 0.21 ^{g}
	3) Cirr.-PH	89.35 ± 40.3 ^{l}	1333.49 ± 340.41 ^{k}	2.82 ± 0.38 ^{i}	0.69 ± 0.01 ^{l}
	4) Cirr.-PH with tt.	140.36 ± 34.8 ^{f}	3999.05 ± 345.07 ^{c}	5.54 ± 0.94 ^{b}	2.65 ± 0.76 ^{e}
	5)PH with tt	121.00 ± 34.6 ^{j}	2132.49 ± 211.07 ^{g}	4.88 ± 0.68 ^{d}	2.75 ± 0.32 ^{e}
1 week	1)Normal	164.67 ± 44.5 ^{c}	1733.15 ± 133.34 ^{i}	11.15 ± 1.41 ^{a}	4.50 ± 0.57 ^{a}
	2)PH	102.00 ± 23.44 ^{k}	1333.46 ± 160.00 ^{k}	6.15 ± 0.34 ^{n}	2.18 ± 0.12 ^{f}
	3) Cirr.-PH	87.73 ± 9.18 ^{l}	1599.66 ± 250.05 ^{j}	4.21 ± 0.16 ^{i}	1.50 ± 0.16 ^{i}
	4) Cirr.-PH with tt.	171.18 ± 30.1 ^{b}	4398.53 ± 360.12 ^{b}	5.77 ± 0.89 ^{b}	3.25 ± 0.91 ^{d}
	5)PH with tt	124.51 ± 30.5 ^{i}	2533.68 ± 200.09 ^{f}	5.21 ± 0.69 ^{ef}	3.18 ± 0.93 ^{d}
2 weeks	1)Normal	164.67 ± 14.5 ^{c}	1733.15 ± 133.34 ^{i}	11.15 ± 1.41 ^{a}	4.50 ± 0.57 ^{a}
	2)PH	101.14 ± 18.45 ^{k}	1599.16 ± 139.27 ^{j}	7.65 ± 0.98 ^{gh}	2.69 ± 0.38 ^{e}
	3)Cirr.-PH.	134.94 ± 18.5 ^{k}	1866.54 ± 349.00 ^{h}	5.32 ± 0.42 ^{e}	1.72 ± 0.03 ^{h}
	4)Cirr.	134.94 ± 30.0 ^{gh}	1333.43 ± 209.00 ^{k}	3.64 ± 0.34 ^{d}	0.94 ± 0.01 ^{k}
	5)Cirr.-PH with tt.	185.44 ± 39.2 ^{a}	4931.30 ± 387.07 ^{a}	10.88 ± 0.87 ^{a}	3.32 ± 0.21 ^{a}
	6)Cirr.with tt	160.45 ± 40.0 ^{c}	3732.84 ± 248.12 ^{d}	5.81 ± 0.57 ^{c}	3.18 ± 0.42 ^{b}
	7)PH with tt	148.22 ± 20.4 ^{e}	2666.63 ± 300.98 ^{e}	10.15 ± 0.66 ^{a}	3.40 ± 0.38 ^{a}

Data are means ± SD of 10 rats in each group. Data are expressed in U/g tissue for catalase enzyme activity and μg/g tissue for glutathione level. Total protein content is expressed as mg/g of liver tissue and albumin level as g/dL Statistical analysis is performed using two way analysis of variance (ANOVA), combined with Co-state computer program and Post hoc (LSD). Unshared letters between groups are significant at p value ≤ 0.05. PH with tt: Partially hepatectomized rats with treatment. Cirr.with tt: Propagated cirrhotic group with treatment. Cirr.-PH with tt.: Propagated cirrhotic- partially hepatectomized rats with treatment .PH: Partially hepatectomized rats. Cirr.: Propagated cirrhotic rats.Cirr.- PH.: Propagated cirrhotic -partially hepatectomized rats

As compared to normal healthy rats, partially hepatectomized rats showed significant (p≤0.05), decrease in catalase enzyme activity and glutathione level, where 1st day post operation demonstrated the most remarkable effect (46.92 and 53.88 % respectively).Gradual increase in catalase activity and glutathione content was detected with intersection of time reaching to their maximum levels post two weeks of surgery, where they recorded significant decrease with percentages amounting to 38.39 and 7.73%, respectively**.** Also, propagated-cirrhotic rats showed significant decrease in catalase enzyme activity (18.05%), and glutathione concentration (23.06%), as compared to normal healthy rats. In addition, cirrhotic-partially hepatectomized rats showed significant (p≤0.05) decrease in catalase activity and glutathione content at 1st day post operation (45.95 and 46.14%, respectively), then gradual increase was noticed with time intersection, exhibited their highest level after two weeks with percentage increase 38.72 and 7.70%, respectively, where they still recorded significant decrease. Considering, partially hepatectomized rats treated with melatonin, significant decrease in the catalase activity was detected, reached to its highest level post two weeks of treatment,

where it still recorded significant decrease with percentage 9.99%. Although, glutathione concentration showed significant increase with time intersection reached to its maximum level post two weeks of treatment (53.86%), as compared to normal healthy rats**.** Regarding, propagated -cirrhotic rats post two weeks of melatonin treatment, insignificant decrease in the catalase enzyme activity was recorded (2.56 %), while, significant increase was detected in glutathione content (115.38%), as compared to normal healthy rats. Moreover, cirrhotic-partially hepactomized rats treated with melatonin demonstrated significant decrease in catalase activity after one and three days of treatment with percentages decrease reached to 16.53 and 14.76%, respectively, followed by significant increase post one and two weeks of treatment (3.96 and 12.62%, respectively). Glutathione level showed significant increase with time intersection reached to its highest level post two weeks of melatonin treatment (184.53%).Thus, it's a great of importance to remark that, treatment with melatonin showed fluctuated improvement percentages in the catalase activity and glutathione content as compared to untreated groups **(Table 2).**

As compared to normal healthy rats, partially hepatectomized rats showed significant (p≤0.05), increase in NO and MDA levels with the highest percentages increase at 1st day post operation (231.59 and 580.65 % respectively), while the lowest percentages increase were detected at 2nd week post operation (125.00 and 300.48%, respectively)**.** Also, propagated-cirrhotic rats showed significant (p≤0.05), increase in NO and MDA levels with percentages increase amounting to 60.50 and 451.41%, respectively, as compared to normal healthy rats. In addition, cirrhotic- partially hepatectomized rats showed significant (p≤0.05), increase in NO and MDA levels recorded the highest levels after one day with percentages increase reached to 119.96 and 1321.39%, respectively, while the lowest percentages increase were recorded post two weeks (94.2 and 278.45%, respectively), as compared to normal control rats. On the other hand, treatment of partially hepatectomized rats with melatonin demonstrated significant increase in the level of NO after one day of treatment with percentage increase of 24.97% followed by insignificant change with intersection of time. However, significant increase was detected in MDA level post melatonin treatment which decreased from 155.4%, after one day of treatment to 127.03%, two weeks post treatment as compared to normal healthy rats**.**

Table 3: Effect of melatonin treatment on nitric oxide and lipid peroxide levels in different therapeutic groups

Time	Groups / Parameters	Nitric oxide	Lipid peroxide
1 Day	1)Normal	5.19 ± 1.65 ^{gh}	65.12 ± 10.65 ^{d}
	2)PH	17.21 ± 6.02 ^{a}	443.24 ± 83.29 ^{c}
	3) Cirr.-PH	11.42 ± 3.06 ^{c}	925.61 ± 90.77 ^{b}
	4) Cirr.-PH with tt.	7.55 ± 2.47 ^{e}	211.29 ± 44.87 ^{a}
	5)PH with tt	6.49 ± 1.56 ^{f}	166.38 ± 25.93 ^{f}
3 Days	1)Normal	5.19 ± 1.65 ^{gh}	65.12 ± 10.65 ^{d}
	2)PH	17.07 ± 6.03 ^{a}	334.82 ± 40.21 ^{f}
	3) Cirr.-PH	11.07 ± 3.03 ^{cd}	869.53 ± 80.87 ^{e}
	4) Cirr.-PH with tt.	6.57 ± 1.07 ^{gh}	301.23 ± 35.56 ^{c}
	5)PH with tt	5.18 ± 1.01 ^{gh}	176.47 ± 34.27 ^{b}
1 week	1)Normal	5.19 ± 1.65 ^{gh}	65.12 ± 10.65 ^{d}
	2)PH	14.68 ± 2.35 ^{b}	265.63 ± 45.46 ^{f}
	3) Cirr.-PH	11.69 ± 1.11 ^{c}	716.35 ± 90.03 ^{e}
	4) Cirr.-PH with tt.	5.54 ± 1.19 ^{fg}	334.52 ± 57.45 ^{c}
	5)PH with tt	4.22 ± 1.00 ^{h}	153.65 ± 35.25 ^{b}
2 weeks	1)Normal	5.19 ± 1.65 ^{gh}	65.12 ± 10.65 ^{d}
	2)PH	11.74 ± 2.33 ^{c}	260.58 ± 43.10 ^{e}
	3)Cirr.-PH.	10.08 ± 2.02 ^{d}	246.45 ± 56.21 ^{h}
	4)Cirr.	8.33 ± 1.74 ^{e}	359.08 ± 58.20 ^{g}
	5)Cirr.-PH with tt.	4.68 ± 1.39 ^{gh}	106.70 ± 33.31 ^{f}
	6)Cirr.with tt	2.96 ± 0.55 ^{i}	293.72 ± 60.60 ^{c}
	7)PH with tt	4.55 ± 1.56 ^{h}	147.84 ± 25.87 ^{b}

Data are means ± SD of 10 rats in each group. Data are expressed as µmol /g tissue for nitric oxide and nmol/g tissue for lipid peroxide. Statistical analysis is performed using two way analysis of variance (ANOVA) combined with Co-state computer program and Post hoc (LSD). Unshared letters between groups are significant at p value ≤ 0.05.PH with tt: Partially hepatectomized rats with treatment. Cirr.with tt: Propagated cirrhotic rats with treatment. Cirr.- PH with tt. : Propagated cirrhotic- partially hepatectomized rats with treatment PH: Partially hepatectomized rats. Cirr.: Propagated cirrhotic rats. Cirr.-PH: Propagated cirrhotic -partially hepatectomized rats.

Treatment of propagated -cirrhotic rats with melatonin demonstrated, significant (p≤0.05), decrease in the NO level (43.07%), while MDA level showed significant increase (351.00%), as compared to normal healthy rats**.** Also, cirrhotic-partial hepactomized treated with melatonin recorded significant increase in NO level after one day of treatment (45.47%), followed by insignificant increase with the time intersection. While, MDA level showed significant increase with the time intersection reached to its lowest level at two weeks post treatment with percentage increase reached to 63.85%.It was obvious that, treatment with melatonin showed significant decrease in the levels of NO and MDA in all treated groups as compared to untreated one **(Table 3)**.

As compared to normal healthy rats, partially hepatectomized rats showed insignificant decrease in AMP level after one, three days and two weeks, while significant decrease (p≤0.05), post one week of operation was demonstrated with percentage decrease reached to 48.45%. ADP level showed significant increase with time intersection reached to its highest percentage increase two weeks post-surgery (1480.00%). However, ATP level showed significant (p≤0.05), decrease with time intersection recorded more or less similar lower levels one and two weeks post operation (72.56% and 71.39%, respectively). Whereas, AEC, recorded significant decrease with time intersection and showed its lowest value two weeks post operation with percentage decrease amounting to 26.23%. Also, TA showed significant decrease at 1st, 3rd day and 1st week with percentages 60.39, 48.69 and 51.52%, respectively, although, two weeks post operation exhibited insignificant change as compared to normal control group. With regard to, inorganic phosphate and phosphate potential, significant decrease (p≤0.05) was noticed in the concentration of inorganic phosphate with different intervals recorded the lowest concentration level after one day (80.20%). While, phosphate potential demonstrated insignificant change after one day, one and two weeks post operation, while significant decrease was observed after three days with percentages decrease of 86.66 and 60.00%, respectively as compared to normal healthy rats. In addition, as compared to normal control rats, propagated -cirrhotic rats showed insignificant decrease in AMP level, while ADP level recorded significant (p≤0.05) increase amounting to 486.39%. In contrast, ATP level demonstrated significant decrease reached to 27.85 %. Moreover, AEC and TA showed insignificant change, while significant (p≤0.05), decrease was detected in inorganic phosphate level and phosphate potential with percentages decrease amounting to 42.86% and 50.00 % as compared to normal healthy rats. Concerning, cirrhotic–partially hepatectomized rats, insignificant change was recorded in AMP level one, two days and one week post operation, while two weeks showed significant increase with percentage increase reached to 118.87 %. While, ADP level exhibited significant increase with the time intersection recorded the highest percentage increase after two weeks (619.00%). In contradictory, ATP showed significant depletion with effect of time, recorded the lowest percentage of decrease after two weeks (45.30%). Regarding to AEC, it showed significant decrease after one, three days and two weeks with percentages 35.80, 11.40 and 18.40%, respectively, while insignificant change in AEC was detected after one week (7.78%). TA and inorganic phosphate showed significant increase (p≤0.05), after one day with percentages increase reached to 79.50 and 38.78%, respectively, while, significant decline was demonstrated in TA after three days (41.80%) and one week (42.80%), followed by insignificant change after two weeks. However, inorganic phosphate level recorded insignificant change after three days and two weeks, while one week showed significant decrease with percentage of 39.52 %, as compared to normal control rats. Furthermore, phosphate potential declared significant decrease after one, three days and two weeks with percentages decrease of 83.6, 83.33 and 73.33%, respectively, while**,** insignificant change was detected after one week**.** Treatment of partially hepatectomized rats with melatonin showed significant increase (p≤0.05) in AMP level at different times with percentages increase reached to 76.40, 71.60, 340.56%, after one, three days and one week post operation respectively except for two weeks which exhibited insignificant change as compared to normal control rats. Also, significant increase was detected in ADP level with the time intersection showed the highest percentage increase after one day (828.97%), while the lowest percentage increase was recorded after two weeks (328.1%). In contrast, ATP level showed significant decrease with the time interaction, recorded the highest percentage decrease after one day (45.67%), while the percentage decrease reached to 10.94%**,** after two weeks, where it still recorded significant decrease as compared to normal control rats. Concerning, AEC, it demonstrated significant decrease post one, three days and one week of melatonin treatment with percentages decrease reached to 13.50, 9.87 and 20.98% respectively, while, insignificant change was observed after two weeks. Whereas, TA and inorganic phosphate recorded insignificant change with the intersection of time. However, phosphate potential demonstrated significant decrease after one and three days with percentages decrease of 66.67 and 76.66%, respectively. While, insignificant change was detected after one week, followed by significant increase (160.00%), post two weeks of melatonin treatment as compared to normal control rats. On the other hand, treatment of propagated-cirrhotic rats with melatonin after two weeks, showed significant increase (p<0.05) in AMP (96.22%) and ADP (79.75%), while ATP, AEC and TA recorded insignificant change as compared to normal control rats. Although, inorganic phosphate showed significant decrease with percentage amounting to 51.60%, whereas significant increase in phosphate potential (156.6%), was observed as compared to normal control rats**.** With respect to, cirrhotic-partially hepactomized rats treated with

melatonin, significant increase in AMP and ADP levels was detected, since AMP showed, its highest level after one day of melatonin treatment with percentage increase of 250.9%, and its lowest value after two weeks with percentage increase reached to 114.15%. Also, ADP recorded its highest value after two weeks with percentage increase 1688.00%, while similar percentage increase was noticed after three days and one week of treatment (659.16%). Whereas, ATP level showed significant decrease with the time intersection, where it recorded percentage decrease amounting to 36.15, 28.42, 28.45 and 23.1%, after one, three days, one and two weeks respectively**.** In addition, AEC showed significant decrease after different durations with percentages decrease reached to 24.69, 14.81, 14.81 and 18.50%, after one, three days, one and two weeks, respectively. However, TA showed significant increase (p≤0.05) after one day and two weeks of melatonin treatment with percentages increase of 31.93 and 54.43%, respectively. While, insignificant increase after three days and one week was detected. Moreover, inorganic phosphate demonstrated significant decrease in cirrhotic-partially hepatectomized rats treated with melatonin with the time intersection as compared to normal control rats, demonstrated percentages decrease of 31.10, 58.45, 59.25 and 75.50%, for one, three days, one and two weeks respectively. Moreover, phosphate potential demonstrated significant (p≤0.05) decrease 83.10% in cirrhotic-partially hepatectomized rats treated with melatonin after one day, while insignificant change was detected after other durations as compared to normal control group **(Tables 4 and 5).**

As compared to normal healthy rats, partially hepatectomized rats showed significant increase (p≤0.05) in TGF-ß, IL-1 and TNF-α levels with the highest percentages after one and two weeks for TGF-ß (64.07 and 63.25%, respectively), and the highest percentages after two weeks for IL-1 and TNF-α (44.36 and 55.00%, respectively), as compared to normal healthy rats. Also, as compared to normal healthy rats, cirrhotic rats showed significant increase (p≤0.05) in TGF-ß, IL-I and TNF-α levels with percentages increase amounting to 68.62, 41.50 and 52.11%, respectively**.** In addition, cirrhotic-partially hepatectomized rats showed significant (p≤0.05), increase in TGF-ß, IL-I and TNF-α, showed the highest level for TGF after three days with percentage increase of 66.70% and after two weeks for IL-1 and TNF-α with percentages increase reached to 46.20 and 56.76%, respectively, as compared to normal control rats**.** On the other hand, treatment of hepatectomized rats with melatonin showed significant increase in the levels of TGF- ß, IL-1 and TNF- α with more or less similar percentages of increase with time intersection, recorded the lowest percentages (5.14, 8.90 and 35.15%, respectively), after two weeks of melatonin treatment as compared to normal control group.

Table 4: Effect of melatonin treatment on AMP, ADP and ATP levels in different therapeutic groups

Time	Gps / Parameters	AMP	ADP	ATP
1 Day	1)Normal	1.06 ± 0.157 ^{gh}	0.57 ± 0.013 ^{jk}	12.03 ± 1.068 ^{a}
	2)PH	0.81 ± 0.02 ^{gh}	1.35 ± 0.014 ^{hi}	3.42 ± 0.001 ^{i}
	3) Cirr.-PH	1.29 ± 0.04 ^{fg}	2.15 ± 0.002 ^{a}	2.43 ± 0.011 ^{j}
	4) Cirr.-PH with tt.	3.72 ± 0.05 ^{b}	7.19 ± 0.012 ^{cd}	7.68 ± 0.501 ^{e}
	5)PH with tt	1.87 ± 0.08 ^{cdef}	5.32 ± 0.276 ^{de}	6.53 ± 0.780 ^{f}
3 Days	1)Normal	1.06 ± 0.157 ^{gh}	0.57 ± 0.013 ^{jk}	12.03 ± 1.068 ^{a}
	2)PH	0.93 ± 0.495 ^{gh}	3.64 ± 0.482 ^{efgh}	2.64 ± 0.490 ^{j}
	3) Cirr.-PH	0.97 ± 0.012 ^{gh}	2.72 ± 0.031 ^{fghi}	4.51 ± 0.203 ^{h}
	4) Cirr.-PH with tt.	2.56 ± 0.011 ^{c}	4.35 ± 0.03 ^{efg}	8.61 ± 0.0018 ^{d}
	5)PH with tt	1.82 ± 1.591 ^{def}	4.97 ± 0.22 ^{def}	7.41 ± 0.889 ^{e}
1 week	1)Normal	1.06 ± 0.157 ^{gh}	0.57 ± 0.013 ^{jk}	12.03 ± 1.068 ^{a}
	2)PH	0.55 ± 0.007 ^{i}	2.98 ± 0.17 ^{efghi}	3.30 ± 0.242 ^{i}
	3) Cirr.-PH	1.39 ± 0.012 ^{efg}	1.31 ± 0.03 ^{hi}	5.35 ± 0.102 ^{g}
	4) Cirr.-PH with tt.	2.56 ± 0.311 ^{c}	4.35 ± 0.05 ^{efg}	8.60 ± 0.003 ^{d}
	5)PH with tt	4.67 ± 0.167 ^{a}	2.86 ± 0.077 ^{fghi}	8.81 ± 0.575 ^{d}
2 weeks	1)Normal	1.06 ± 0.157 ^{gh}	0.57 ± 0.013 ^{jk}	12.03 ± 1.068 ^{a}
	2)PH	0.80 ± 0.567 ^{gh}	9.06 ± 0.903 ^{bc}	3.44 ± 0.201 ^{i}
	3)Cirr.-PH.	2.31 ± 0.891 ^{cd}	4.12 ± 0.383 ^{efg}	6.58 ± 0.219 ^{f}
	4)Cirr.	0.73 ± 0.06 ^{gh}	3.36 ± 0.982 ^{efgh}	8.68 ± 0.801 ^{d}
	5)Cirr.-PH with tt.	2.27 ± 0.976 ^{cd}	10.25 ± 1.760 ^{b}	9.24 ± 0.65 ^{c}
	6)Cirr.with tt	2.08 ± 0.043 ^{cde}	1.03 ± 0.021 ^{i}	11.96 ± 0.805 ^{a}
	7)PH with tt	1.20 ± 0.213 ^{fgh}	2.45 ± 0.811 ^{gh}	10.71 ± 0.474 ^{b}

Data are means ± SD of 10 rats in each group. Data are expressed µmol /g wet weight. Statistical analysis is performed by using two way analysis of variance (ANOVA) combined with Co-state computer program and Post hoc (LSD) .Unshared letters between groups are significant at p value ≤ 0.05. PH with tt: Partially hepatectomized rats with treatment. Cirr.with tt: Propagated cirrhotic rats with treatment. Cirr.- PH . with tt : Propagated cirrhotic-partially hepatectomized rats with treatment. PH: Partially hepatectomized rats. Cirr.: Propagated cirrhotic rats.Cirr.-PH.: Propagated cirrhotic -partially hepatectomized rats.

Table 5: Effect of melatonin treatment on adenylate energy charge, total adenylate, inorganic phosphate levels and phosphate potential in different therapeutic groups

Time	Groups / Parameters	AEC	TA	Inorganic phosphate	Phosphate potential
1 Day	1)Normal	0.81 ± 0.02 ab	14.09 ± 0.71 de	31.05 ± 0.002 bc	0.30 ± 0.0072 bc
	2)PH	0.73 ± 0.02 cd	5.58 ± 0.81 f	6.12 ± 0.03 ij	0.41 ± 0.003 b
	3) Cirr.-PH	0.52 ± 0.01 g	25.30 ± 0.91 a	43.09 ± 2.76 a	0.049 ± 0.001 e
	4) Cirr.-PH with tt.	0.61 ± 0.01 f	18.59 ± 0.31 c	21.39 ± 0.23 defg	0.05 ± 0.001 e
	5)PH with tt	0.70 ± 0.12 cde	13.72 ± 5.81 de	24.02 ± 3.07 cdef	0.10 ± 0.0855 d
3 Days	1)Normal	0.81 ± 0.02 ab	14.09 ± 0.11 de	31.05 ± 0.002 bc	0.30 ± 0.0072 bc
	2)PH	0.64 ± 0.10 ef	7.23 ± 2.49 f	10.63 ± 0.96 f	0.40 ± 0.001 cd
	3) Cirr.-PH	0.72 ± 0.08 cd	8.20 ± 0.04 f	31.68 ± 0.087 b	0.05 ± 0.678 e
	4) Cirr.-PH with tt.	0.69 ± 0.02 cde	15.90 ± 0.05 de	12.90 ± 0.08 hi	0.25 ± 0.003 bc
	5)PH with tt	0.73 ± 0.15 cd	14.21 ± 4.93 de	26.20 ± 5.74 bcde	0.07 ± 0.001 d
1 week	1)Normal	0.81 ± 0.02 ab	14.09 ± 0.11 de	31.05 ± 0.002 bc	0.30 ± 0.0072 bc
	2)PH	0.72 ± 0.08 cd	6.83 ± 2.75 f	15.57 ± 6.931 gh	0.23 ± 0.0018 bc
	3) Cirr.-PH	0.75 ± 0.01 bc	8.05 ± 0.05 f	18.78 ± 0.87 fgh	0.22 ± 0.003 bc
	4) Cirr.-PH with tt.	0.69 ± 0.01 cde	15.55 ± 0.03 de	12.65 ± 0.04 hi	0.26 ± 0.005 bc
	5)PH with tt	0.64 ± 0.08 ef	16.28 ± 3.67 cd	32.34 ± 1.112 b	0.25 ± 0.131 bc
2 weeks	1)Normal	0.81 ± 0.02 ab	14.09 ± 0.11 de	31.05 ± 0.002 bc	0.30 ± 0.0072
	2)PH	0.60 ± 0.08 f	13.30 ± 0.07 e	19.77 ± 0.78 efg	0.27 ± 0.005 b
	3)Cirr.-PH.	0.66 ± 0.01 def	13.02 ± 1.28 e	24.26 ± 4.581 cdef	0.08 ± 0.004 e
	4)Cirr.	0.81 ± 0.09 ab	12.76 ± 0.01 e	17.74 ± 0.67 fgh	0.15 ± 0.007 d
	5)Cirr.-PH with tt.	0.66 ± 0.06 def	21.76 ± 0.01 b	7.60 ± 0.45 i	0.27 ± 0.008 bc
	6)Cirr.with tt	0.83 ± 0.04 a	15.08 ± 0.05 de	15.02 ± 0.89 gh	0.77 ± 0.067 a
	7)PH with tt	0.83 ± 0.03 a	14.37 ± 0.56 de	27.81 ± 8.065 bcd	0.78 ± 0.042 a

Data are means ± SD of 10 rats in each group. Inorganic phosphate is expressed as µmol /g wet weight. Adenylate energy charge (AEC) and phosphate potential are without dimension. Statistical analysis is performed using two way analysis of variance (ANOVA) combined with Co-state computer program and Post hoc (LSD). Unshared letters between groups are significant at p value ≤ 0.05.PH with tt: Partial hepatectomized rats with treatment. Cirr.with tt: Propagated cirrhotic rats with treatment. Cirr. -PH. with tt : Propagated cirrhotic- partially hepatectomized with treatment. PH: Partially hepatectomized rats. Cirr.: Propagated cirrhotic rats. Cirr.-PH: Propagated cirrhotic -partially hepatectomized rats.

Treatment of propagated-cirrhotic rats with melatonin after two weeks, showed significant increase in the level of TGF-ß, IL-1 and TGF-α with percentages increase of 7.64, 18.35 and 33.69 %, respectively, as compared to normal healthy rats**.** With respect to, cirrhotic - hepatectomized rats treated with melatonin, significant increase in TGF-ß, IL-1 and TNF-α was detected at different durations of treatment recorded matched percentages increase**.** However the lowest percentages for TGF-ß and IL-1 were noticed post one day and two weeks of melatonin treatment (16.59 and 33.00%, respectively for TGF- ß, 13.80 and 17.50 %, respectively for IL-1)**,** as compared to normal healthy rats. Also, TNF-α recorded the lowest percentages one and two weeks post treatment (22.38 and 22.44%, respectively)**.** From the manipulated data, treatment with melatonin demonstrated percentages of improvement in the levels of TGF- ß, IL-1, TNF-α in all treated groups as compared to untreated ones **(Table 6).**

As compared to normal healthy rats, partially hepatectomized rats showed significant increase (p≤0.05) in EGF and HGF levels with the highest percentages increase after one and two weeks for EGF recorded 45.96 and 46.83%, respectively. HGF demonstrated the highest percentages increase after one and three days post operation (6.51 and 6.10 %, respectively). Significant decrease was noticed in HGF level post two weeks of operation with percentages decrease reached to 3.61%, Also, propagated-cirrhotic rats after two weeks, recorded significant (p≤0.05), increase in EGF and HGF with percentages increase reached to 44.30 and 25.80%, respectively as compared to normal control rats**.** In addition, cirrhotic–partially hepatectomized rats demonstrated significant increase in EGF with the time intersection showed more or less similar percentages increase after one, three days, one and two weeks (39.40, 41.9, 46.76 and 46.74%, respectively). Whereas HGF showed significant increase (16.25%), post one day of operation, followed by insignificant change after three days, one and two weeks (2.92, 3.60 and 0.40%, respectively), as compared to normal control groups. With respect to, treatment of hepatectomized rats with melatonin, significant increase in EGF was noticed after one and three days post treatment with percentages increase reached to 4.30 and 4.50 %, respectively, followed by significant decrease post one and two weeks of melatonin treatment with percentages 8.70 and 7.40 %, respectively. While, HGF showed, significant increase with the time intersection with percentages increase

reached to 16.03, 16.06, 19.10 and 12.70%, respectively after one, three days, one and two weeks of melatonin treatment as compared to normal healthy rat. As compared to normal control group, treatment of propagated -cirrhotic rats with melatonin (after two weeks), recorded, significant decrease in the levels of EGF 3.90 %, while HGF demonstrated insignificant change. Moreover, cirrhotic-hepatectomized rats treated with melatonin, showed significant decrease in EGF level after one day and two weeks ($p \leq 0.05$), with percentages decrease amounting to 8.01, 4.09%, respectively, while three days and one week recorded significant increase reached to 8.01 and 38.60%, respectively. Also, HGF level declared, significant increase after one, three days, one and two weeks of melatonin treatment with percentages increase of 13.80, 12.30, 18.60 and 18.50%, respectively as compared to normal control rats Remarkable enhanced effect of melatonin was detected in the levels of EGF and HGF in all treated groups as compared to untreated one **(Table 6)**.

Table 6: Effect of melatonin treatment on TGF- ß, IL-1, TNF- α, EGF and HGF

Time	Groups / Parameters	TGF-β	IL-1	TNF	EGF	HGF
1=]] Day	1)Normal	36.52±2.01^{r}	54.21±3.55^{q}	61.52±2.02^{r}	50.41±7.55^{j}	31.50±3.35^{n}
	2)PH	51.36±6.01^{j}	74.03±9.02^{g}	90.25±10.02^{g}	71.69±7.00^{e}	33.55±2.12^{j}
	3) Cirr.-PH	57.05±3.03^{f}	72.40±7.60^{i}	92.58±7.44^{e}	70.29±6.02^{f}	36.62±3.02^{e}
	4) Cirr.-PH with tt.	42.58±4.03^{l}	61.73±4.23^{o}	87.33±9.00^{j}	46.37±4.02^{m}	35.87±0.88^{g}
	5)PH with tt	39.50±6.01^{n}	70.21±5.33^{j}	85.60±6.05^{l}	52.58±2.04^{i}	36.55±0.92^{f}
3 Day	1)Normal	36.52±2.01^{r}	54.21±3.55^{q}	61.52±2.02^{r}	50.41±7.55^{j}	31.50±3.35^{n}
	2)PH	56.89±3.02^{h}	74.91±4.02^{f}	89.92±5.04^{h}	71.91±7.03^{d}	33.41±3.02^{k}
	3) Cirr.-PH	60.88±4.65^{b}	75.89±8.49^{e}	92.55±6.03^{f}	71.55±3.03^{e}	32.42±2.01^{n}
	4) Cirr.-PH with tt.	55.90±4.01^{i}	72.92±7.03^{h}	88.93±4.02^{i}	69.90±6.81^{g}	35.40±5.66^{i}
	5)PH with tt	39.60±2.03^{m}	70.23±6.43^{j}	85.63±6.30^{k}	52.68±3.43^{i}	36.75±7.02^{d}
1 week	1)Normal	36.52±2.01^{r}	54.21±3.55^{q}	61.52±2.02^{r}	50.41±7.55^{j}	31.50±3.35^{n}
	2)PH	59.92±5.01^{c}	76.12±6.66^{d}	95.29±7.63^{c}	73.58±3.03^{b}	32.36±1.02^{m}
	3) Cirr.-PH	59.62±6.32^{d}	78.26±7.61^{b}	95.44±8.66^{b}	73.98±6.23^{a}	30.36±2.02^{n}
	4) Cirr.-PH with tt.	56.82±4.03^{g}	66.12±4.52^{l}	75.29±5.43^{q}	68.58±8.04^{h}	37.36±7.32^{c}
	5)PH with tt	38.55±3.02^{p}	69.74±5.32^{k}	85.05±3.23^{m}	45.98±5.60^{n}	37.53±6.22^{b}
2 weeks	1)Normal	36.52±2.01^{r}	54.21±3.55^{q}	61.52±2.02^{r}	50.41±7.55^{j}	31.50±3.35^{n}
	2)PH	59.78±5.93^{d}	78.26±8.01^{b}	95.44±6.04^{b}	74.02±5.06^{a}	30.36±3.22^{q}
	3)Cirr.-PH.	58.62±5.42^{e}	79.26±7.02^{a}	96.44±6.05^{a}	73.97±4.94^{a}	31.36±3.32^{n}
	4)Cirr.	61.58±2.73^{a}	76.74±6.02^{c}	93.58±3.02^{d}	72.78±5.83^{c}	39.65±5.23^{a}
	5)Cirr.-PH with tt.	48.58±4.82^{k}	63.73±5.01^{n}	75.33±5.04^{p}	48.35±2.02^{k}	37.34±7.69^{c}
	6)Cirr.with tt	39.31±2.02^{o}	64.16±3.03^{m}	82.25±7.04^{o}	48.44±3.78^{k}	30.42±4.32^{n}
	7)PH with tt	38.40±4.31^{q}	59.04±4.03^{p}	83.15±6.03^{n}	46.64±4.03^{l}	35.53±5.02^{h}

Data are means ± SD of 10 rats in each group. Data are expressed in pg/mL. Statistical analysis is performed using two way analysis of variance (ANOVA) combined with Co-state computer program and Post hoc (LSD) .Unshared letters between groups are significant at p value ≤ 0.05. PH with tt: Partially hepatectomized rats with treatment. Cirr.with tt: Propagated cirrhotic rats with treatment. Cirr.-PH with tt.: Propagated cirrhotic- partially hepatectomized with treatment. PH: Partially hepatectomized rats. Cirr.: Propagated cirrhotic rats. Cirr.-PH: Propagated cirrhotic -partially hepatectomized rats

Histopathological examination

Liver section from Partial Hepatectomized rats after one day (Fig.7, b) showed moderate hydropic degeneration with moderate lymphocytic infiltration in between hepatocytes and moderate sinusoidal dilatation, also after 3 days (Fig.7, c) showed moderate hydropic degeneration with focal lymphocytic infilteration between hepatocytesm. While after one week and two weeks (Fig.7, d, e), almost normal hepatocytes arranged in thin plates with mild sinusoidal dilation were detected. In addition, liver section from propagated cirrhotic-partial hepatectomized rats after 1 day (Fig.7, f) showed congestion of central vein and vacuolar degeneration and post 3 days (Fig.7, g) showed moderate fibrous tissue in portal tract, moderate hydropic degeneration, moderate ballooning, moderate lymphocytic infiltration of portal tract propagated cirrhotic-partial hepatectomized.

However after 1 week mild fibrous tissue in portal tract, moderate hydropic degeneration, moderate lymphocytic infiltration of portal tract were seen (Fig.7, h), after 2 weeks showing damage in the hepatic architecture, congestion of portal tract, infiltrative inflammatory cells and necrosis. Also, cirrhotic rats showed moderate fibrous tissue in portal tract III, moderate hydropic degeneration, moderate ballooning, moderate lymphocytic infiltration of portal tract (Fig.7, i). Partial

hepatectomized rats treated with melatonin for 1 day showed mild hydropic degeneration, melatonin treatment for 3 days showed mild lymphocytic infiltration in between hepatocytes, treatment for 1 week and 2 weeks showed normal hepatocytes, mild hydropic degeneration (Figs.7, j-m). While, cirrhotic rats treated with Melatonin for 2 weeks showed moderate fibrous tissue II in portal tract, moderate hydropic degeneration, moderate lymphocytic infiltration of portal tract (Fig.7, n, o). Propagated cirrhotic-partial hepatectomized rats treated with Melatonin for 1 day showed moderate fibrous tissue II in portal tract, moderate hydropic degeneration, moderate lymphocytic infiltration of portal tract (Fig.7, p). Also post 3 days of Melatonin treatment focal fibrous tissue in portal tract I, mild hydropic degeneration with focal lymphocytic infiltration of portal tract were observed (Fig.7, q). While after 1 week of Melatonin treatment, damage in the hepatic architecture and vacuolar degeneration were detected (Fig.7, r). Although, post 2 weeks of treatment no fibrous tissue in portal tract, mild hydropic degeneration, mild lymphocytic infiltration of portal tract were noticed (Fig.7, s).

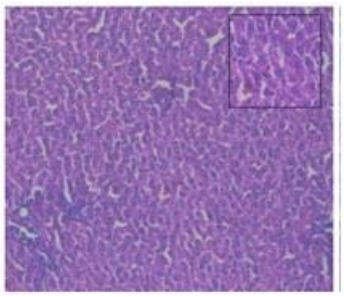

Fig.7,a) Liver section from normal rats showing normal hepatocytes arranged in thin plates (H&E,x200)

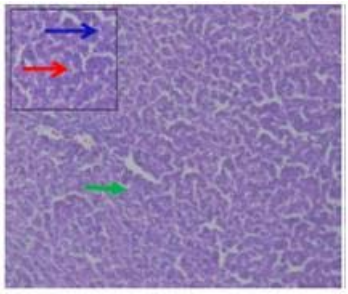

Fig.7,b) Liver section from partial hepatectomized rats after one day showing moderate hydropic degeneration (red arrow) with moderate lymphocytic infiltration in between hepatocytes (blue arrow) and moderate sinusoidal dilatation (green arrow) (H&E,x200)

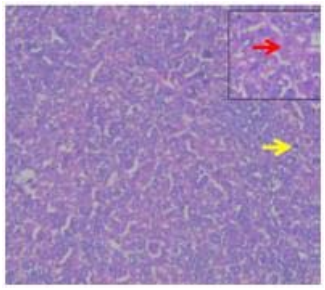

Fig.7,c) Liver section partial hepatectomized rats after three days showing moderate hydropic degeneration (red arrow) with focal lymphocytic infilteration between hepatocytes (yellow arrow) (H&E,x200)

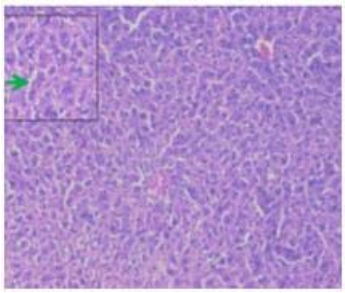

Fig.7,d) Liver section from partial hepatectomized rats after one week showing almost normal hepatocytes arranged in thin plates with mild sinusoidal dilation (green arrow) (H&E,x200)

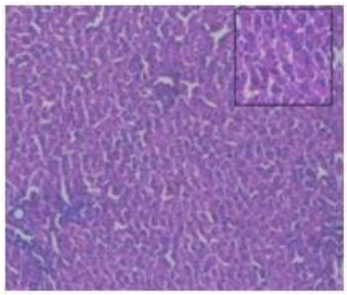

Fig.7,e) Liver section from partial hepatectomized rats after two weeks showing almost normal hepatocytes arranged in thin plates (H&E,x200)

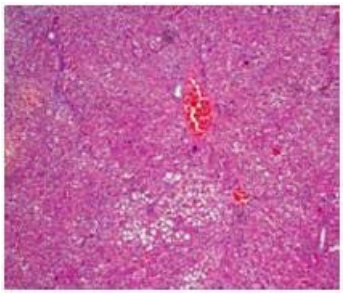

Fig.7,f) Liver section from propagated cirrhotic-partial hepatectomized rats after one day showing congestion of central vein and vacuolar degeneration

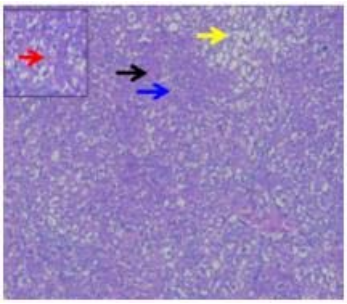

Fig.7,g) Liver section from propagated cirrhotic-partial hepatectomized rats after three days showing moderate fibrous tissue in portal tract (black arrow), moderate hydropic degeneration (red arrow), moderate ballooning (yellow arrow) moderate lymphocytic infiltration of portal tract (blue arrow) (H&E,x200).

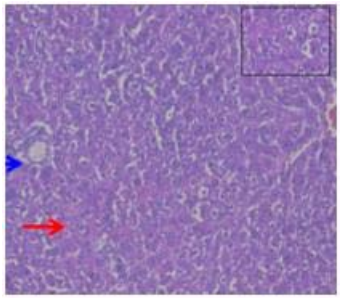

Fig.7,h) Liver section from propagated cirrhotic-partial hepatectomized after one week showing mild fibrous tissue in portal tract, moderate hydropic degeneration (red arrow), moderate lymphocytic infiltration of portal tract (blue arrow) (H&E,x200).

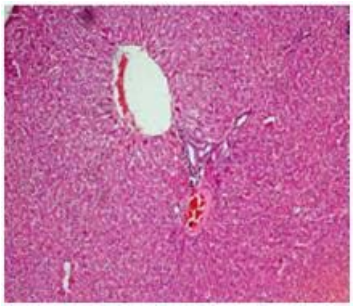

Fig.7,i) Liver section from propagated cirrhotic-partial hepatectomized after two weeks showing damage in the hepatic architecture, congestion of portal tract, infiltrative inflammatory cells and necrosis

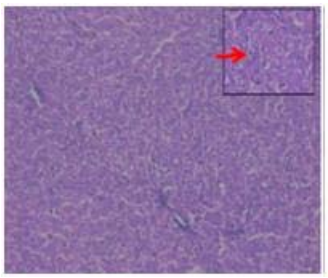

Fig.7,j) Liver section from partial hepatectomized rats treated with melatonin for one day showed mild hydropic degeneration (red arrow) (H&E,x200)

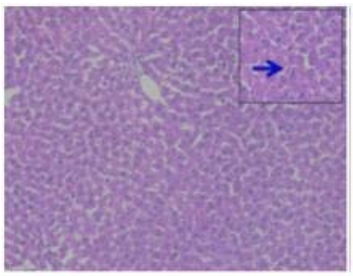

Fig.7,k) Liver section from partial hepatectomized treated with melatonin for three days showing mild lymphocytic infiltration in between hepatocytes (blue arrow) (H&E,x200)

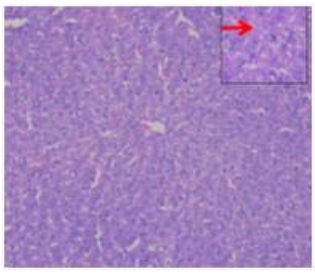

Fig.7,l) Liver section from partial hepatectomized rats treated with melatonin for one week showing mild hydropic degeneration (red arrow) (H&E, x200)

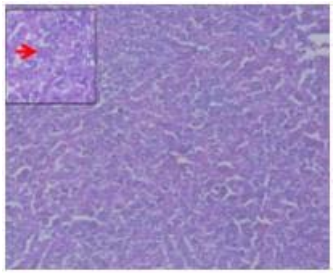

Fig.7,m) Liver section from partial hepatectomized rats treated with melatonin for two weeks showing mild hydropic degeneration (red arrow) (H&E,x200)

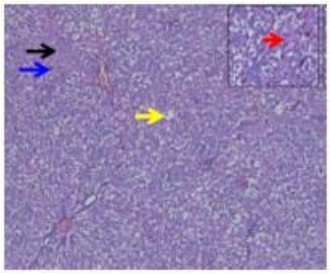

Fig.7,n) Liver section from cirrhotic rats only showing moderate fibrous tissue in portal tract III (black arrow), moderate hydropic degeneration (red arrow), moderate ballooning (yellow arrow) moderate lymphocytic infiltration of portal tract (blue arrow) (H&E,x200).

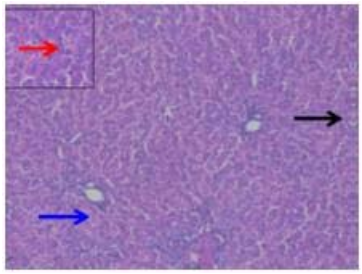

Fig.7,o) Liver section from cirrhotic rats treated with melatonin for two weeks showing moderate fibrous tissue II in portal tract (black arrow), moderate hydropic degeneration (red arrow), moderate lymphocytic infiltration of portal tract (blue arrow) (H&E,x200).

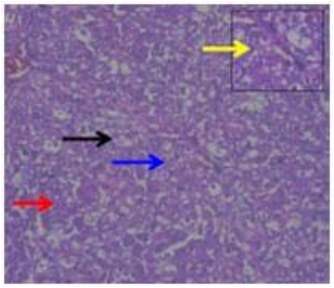

Fig.7,p) Liver section from from propagated cirrhotic-partial hepatectomized treated with melatonin for one day showing moderate fibrous tissue II in portal tract (black arrow), moderate hydropic degeneration (red arrow), moderate lymphocytic infiltration of portal tract (blue arrow) (H&E,x200).

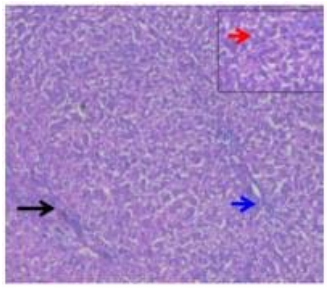

Fig.7,q) from propagated cirrhotic-partial hepatectomized treated with melatonin for three days showing focal fibrous tissue in portal tract I (black arrow), mild hydropic degeneration (red arrow)with focal lymphocytic infiltration of portal tract (blue arrow) (H&E, x200)

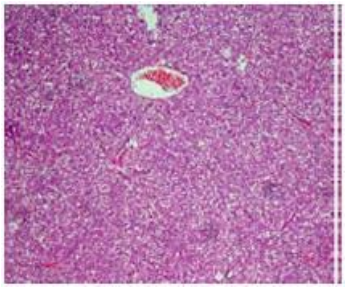

Fig.7,r) Liver section from propagated cirrhotic-partial hepatectomized treated after one week treatment with melatonin showing damage in the hepatic architecture andvacuolar degeneration

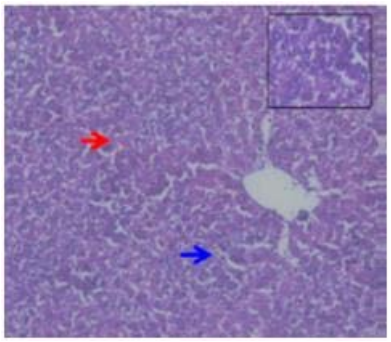

Fig.7,s) Liver section from from propagated cirrhotic-partial hepatectomized treated with melatonin for two weeks showing no fibrous tissue in portal tract ,mild hydropic degeneration (red arrow), mild lymphocytic infiltration of portal tract (blue arrow) (H&E,x200).

DISCUSSION

Liver injury is characterized by oxidative stress, inflammation and fibrosis. The use of drugs for cirrhosis treatment might be focused to attack the causes of oxidative stress and inflammation preventing cirrhosis complications and apoptosis.[21]

Because of liver function is usually impaired in patients with cirrhosis, and because cirrhotic livers are less able to regenerate, it is important to stimulate both the re-generation and function of the remnant cirrhotic liver after hepatectomy. In the present study, we evaluate whether melatonin is effective in stimulating liver regeneration post 33% partial hepatectomy, DMN induced propagation of cirrhosis, and both. The present results clearly demonstrate, significant increase in liver function enzyme activities and total bilirubin level, while significant decrease in total protein content and albumin level in partial hepatectomized, cirrhotic and cirrhotic–partially hepatectomized rats with time intersection.

The differential diagnosis of specific liver diseases is aided by the measurement of aspartate (AST) and alanine (ALT) amino transferases levels. These amino transferases are enzymes involved in the reactions of nitrogen removal from amino acids (transamination reaction). In addition, such enzymes also known as important biomarkers of hepatic function, specially ALT[55, 56].

Monitoring ALT and AST in plasma has been reported to be sensitive indicators of liver injury[57]. The obtained results regarding to the effect of DMN intoxication on the concentrations of ALT and AST are in agreement with those reported studies of Opoku et al.[58] and Gowri et al.[59] The elevation of plasma AST and ALT activities could be regarded as an index of the liver parenchymal cells damage[59]. Bilginet al.[60] attributed the increase in serum enzyme levels to damage of the structural integrity of liver which lead to enzymes release into the circulation; a process of cytolysis. Also, Liver enzymes (AST, ALT and ALP) were elevated after 24 hours of partial hepatectomy and that is in agreement with the results of Takeda et al.[13].

It's well known that, DMN-induced liver injury in rats which is a reproducible and potentially valuable animal model for studying human hepatic cirrhosis. Detoxification of DMN is takes place in the liver by the microsomal cytochrome P450 IIE1 group of enzymes. Apoptosis is considered as a mechanism for cell death in DMN-induced liver injury. It induced apoptosis similar to that found in human beings with hepatic fibrosis and alcoholic cirrhosis[38]. Moreover Ozawa et al.[61] and Hassan and Yousef[62] found that, rats received nitrosamine precursors showed significant increase in serum total bilirubin, and liver function enzyme activities ;AST, ALT and ALP in both serum and liver. The increased level in total bilirubin in the serum of nitrosamine precursors-treated rats could be attributed to the increase of red blood corpuscles destruction in the rate and/or damage of the liver tissue. In addition, hyperbilirubinaemia is a result of severe haemolysis, impaired secretion of bilirubin or cholestasis[63].

With respect to total protein content and albumin level, the current results demonstrated significant decrease in total protein content in all experimental untreated groups. This observation is in accordance to the results of Thirunavuk karasu et al.[64] and Wang et al.[65] as they found that, liver cirrhosis is accompanied by a fall in whole-body protein turnover and consequently albumin level. The reduction in the total protein content and albumin level in the liver during DMN administration may be attributed to the increase in amino acids deamination and impairment in cellular proteins construction [66]. In addition, the liver is the sole source of the plasma proteins bulk mainly albumin, fibrinogen, and prothrombin. Most of a and b globulins are also of hepatic origin, thus the low protein level may be ascertained perturbation in protein synthetic machinery in the liver[67]. Furthermore, the lower serum albumin and protein levels that observed in nitrosamine precursors-treated group might be also due to the formation of the toxic N-nitroso compounds. The inhibition of oxidative phosphorylation process caused by N-nitrosamines (NAs) may lead to this decrease in serum protein content and albumin level as mentioned by Anthony et al.[68] who reported that sodium nitrite decreased total serum protein content and albumin mainly through its effect on the liver by inhibiting oxidative phosphorylation process, and hence the availability of the energy source of protein synthesis and other metabolic processes. In addition, The deterioration of the liver function by sodium nitrites produce retardation of growth, necrotic changes of the liver and deterioration of the liver function giving this disturbance in protein metabolism[68, 69]. The generation of free radicals, increased LPO, and decreased antioxidants are considered to play a vital role in the toxic effects of nitrosamine compounds[70].

The process of regenerating liver is the result of a balance between stimulating factors and inhibitors of hepatocyte proliferation. Melatonin and its metabolites have been found to protect tissues against oxidative damage generated by a variety of toxic agents and metabolic processes. Furthermore, studies in liver of rats showed a decrease in the liver mitochondrial hydroxylation of drugs returning to the normal state after the administration of antioxidants.[72] Partial hepatectomy significantly reduced the biliary flow (36%), and promoted oxidative stress with an increase of lipoperoxidation and decrease of glutathione peroxidase and catalase activities. Treatment with melatonin prevented the decrease of biliary flow in rats with hepatectomy and normalized the Na^+/K^+ATPase activity.[73] Moreover, melatonin markedly attenuated oxidative stress produced by partial hepatectomy. These amelioration signs of melatonin can be explained on the basis of, melatonin prevents the damage of membrane proteins susceptible to be attacked by the ROS, thus preserved membrane permeability and lockage of liver

enzymes into the circulation[71, 72, 73]. Within the compensating systems melatonin can be capable of preventing oxidative damage, through non-enzymatic and enzymatic cellular mechanisms. Among the non-enzymatic cellular mechanisms, GSH content can have important role in various biological phenomena such as the detoxification of electrophilic metabolites of xenobiotics and protection against free radicals[74]. It was suggested that oxidative stress before and during liver regeneration has a crucial role in cholestasis, apoptotic/necrotic hepatocellular damage and the impairment in liver transport function induced by partial hepatectomy, and that melatonin could modulate the degree of oxidative stress and through it prevent the alterations in liver function carrier[74].

Melatonin has a very potent antioxidant activity, depending mainly on its capacity to act as an electron donor[75, 76]. *In vivo* and *in vitro*, melatonin has been known as a radical scavenger with the ability to remove ROS and reactive nitrogen species (RNS). The interaction of melatonin with ROS/ RNS is a prolonged process that involves many of its metabolites. This reaction is a novel property of melatonin and explains how it differs from other conventional antioxidants. Melatonin and its metabolites have been found to protect tissues against oxidative damage generated by a variety of toxic agents and metabolic processes[75, 77]. Furthermore, studies on rats liver showed a decrease in the liver mitochondrial hydroxylation of drugs returning to normal after the administration of antioxidants [74, 78].

The current results markedly showed that, treatment of all experimental groups with melatonin showed fluctuated amelioration percentages associated with significant decrease in the liver enzyme activities and total bilirubin level in all treated groups as compared to untreated one. Also, hepatectomized rats treated with melatonin showed normalization in total protein content and albumin level after two weeks of treatment, with percentages of improvement by 22.41 and 15.77%, respectively. Also, cirrhotic rats demonstrated percentages of amelioration in both total protein content and albumin level reached to 10.49 and 49.77%, respectively. Moreover, cirrhotic- partially hepactomized rats, treated with melatonin recorded percentages of improvement in total protein content and albumin level reached to 54.77 and 35.48%, respectively after two weeks of melatonin treatment.

In agreement with the present results Gonzalez et al.[74] found that, during liver regeneration, increase in some liver enzymes occurred and that treatment with antioxidants such as melatonin may protect the liver against oxidative damage *via* enzymatic mechanisms. This was evidenced in our work by normalizing liver function enzymes in the partial hepatectomized rats treated with melatonin. Also Jung et al.,[28] found that, decreased levels of AST, ALT, ALP, T-bilirubin, and increased levels of albumin and total protein in the melatonin-treated rats as compared to the DMN-induced liver injury may be due to antioxidant effect of melatonin, that preserved plasma membrane and enhanced protein structure in liver. Treatment with melatonin significantly attenuated the increased level of serum amino transferase, which is in parallel results with Hu et al.[79] who found that melatonin is capable to reduce the severe extent of hepatic cell damage in different acute and chronic liver injuries, steatosis and the immigration of inflammatory cells. Also, Kireev et al.[80] observed that melatonin, given before the induction of ischemia/reperfusion significantly attenuated the elevations in serum liver transaminases (ALT/AST), decreased coagulation necrosis and average of steatosis. Our findings are in accordance also with the previous study of Ohta et al.[81], revealed administration of the pineal hormone appeared to reduce the rise in serum enzyme levels of AST and ALT, after almost all types of hepatic injury, indicating that the extent of cell damage was reduced.

In this concern, Meki et al.,[82] showed that, melatonin treatment enhanced hepatic antioxidant/ detoxification systems consequently reducing the apoptotic rate and necrobiotic changes in the liver. Flow cytometric analysis showed that melatonin slowed cell cycle progression by increasing the number of cells in G0 and G1 phases. Jouet al.,[83] observed that, melatonin readily rescued mitochondria from oxidative stress-induced dysfunction, effectively preventing subsequent apoptotic events and death in rat brain astrocytes (RBA-1). So it seems that, melatonin prevents mitochondria-mediated apoptosis in various diseases[83]. In addition, melatonin exert antiproliferative and proapoptotic actions on a number of different cancer cell lines, through diverse mechanisms, including antioxidant effect enzymes, growth factor/hormone receptor binding, and direct or indirect interactions with nucleic acids [84, 85]. Thus, although acute liver injury, melatonin triggered liver regeneration, in the present study there is enhanced restoration to the normal level after partial hepatectomy as compared to untreated partial hepatectomized rats. This effect may depend on the mechanisms of the remodeling process after 33% partial hepatectomy, or dose -dependent of melatonin on the remaining liver as antiproliferative agents [86].

Considering partially hepatectomized rats treated with melatonin, significant decrease in the catalase enzyme activity at 1st 3rd days and one week post treatment, while insignificant change was detected two weeks post treatment. Although, glutathione concentration showed significant increase with time intersection reaching to its maximum level post two weeks of treatment with percentage of improvement 61.59%. Also, treatment of propagated-cirrhotic rats with melatonin recorded percentages of amelioration 15.589 and 138.44 %, for catalase enzyme activity and glutathione level respectively. Moreover, cirrhotic- partially hepatectomized rats treated with melatonin demonstrated significant increase in catalase enzyme

activity post one and two weeks of treatment with percentages of enhancement reached to 50.678 and 51.33 %, respectively. While, glutathione level showed significant increase with time intersection reached to its highest level post two weeks of melatonin treatment with percentage of amelioration of 176.832 %.

Thus, it's a great of importance to mention that treatment with melatonin showed fluctuated improvement percentages in the catalase activity and glutathione content.

Moreover, treatment of partially hepatectomized rats with melatonin showed insignificant change in NO level after three days, one and two weeks while, significant increase was detected after 1st day. However, the percentages of enhancement in MDA level post melatonin treatment reached to 214.5%. In addition, propagated -cirrhotic rats treated with melatonin demonstrated, percentages of amelioration reached to 103.4 % and 100.4 %, respectively for NO and MDA levels. Also, cirrhotic-partially hepatectomized rats treated with melatonin recorded percentages of improvement in NO level reached to 103.7% after two weeks of treatment. While, MDA level showed significant increase with time intersection reached to its lowest level after two weeks of melatonin treatment with percentage of improvement reached to 214.5%.

Treatment with melatonin is a dose-dependent and it reduced lipid peroxide content in carbon tetrachloride treated rats. This indicated that, melatonin exerts a therapeutic effect on carbon tetrachloride-induced acute liver injury in rats, possibly through its antioxidant action[87]. It was found that, melatonin protect the liver in several models of injury *via* the inhibition of oxidative and nitrosative damages and pro-inflammatory markers. Such as, melatonin inhibited the activity of inducible NO synthase, reducing NO formation, and was also able to prevent lipid peroxidation induced hepatotoxicity in endotoxemic rats [80, 88].

In a parallel results, Rodriguez-Reynoso[89] and Okatani et al.,[90] found that, melatonin was able to decrease the expression of iNOS observed in the livers of rats submitted to ischemia/reperfusion and thus to reduce the amount of NOx metabolites. The authors added that melatonin administration was able to reduce plasma nitrite level and iNOS mRNA expression in the liver, which peaked at 2 h of reperfusion in Sprague-Dawley rats. However, Zhang et al.[91] found that, melatonin increased hepatic NO levels for up to 12 h after reperfusion. It is possible that, melatonin could react with ONOO and by this way it can regulate the level of nitrosative stress [92]. It has been reported that apoptosis of hepatocytes and sinusoidal endothelial cells is a critical mechanism contributing to the hepatic ischemia/reperfusion injury [93]. Higher concentrations of toxic products such as $ONOO^-$ or other reactive oxygen species may lead to cell necrosis and apoptosis[94]. So, Suzuki et al. [95]found that the steatotic liver is more vulnerable to ischemia/reperfusion injury mainly due to hepatocyte apoptosis associated with the early up-regulation of iNOS-related peroxinitrite, which in turn leads to subsequent necrosis.

The powerful antioxidant capacity of melatonin has been usually attributed to its potential to scavenge free radicals by the donation of electrons[96]. In addition to, these direct interactions with reactive oxygen species, melatonin may also induce an up-regulation of the activity of antioxidants and antioxidant enzymes as glutathione peroxidase (GPx) and glutathione transferase (GST) in the cytosol fraction of damaged livers, and this can be potentiated in an environment of oxidative stress[26]. Treatment of partially hepatectomized rats with melatonin showed significant ($p \leq 0.05$), increase in AMP level at different times except for two weeks which exhibited an insignificant change as compared to normal control rats. Also, significant increase was detected in ADP level with the time intersection showed the highest percentage increase after one day with percent of improvement 693.023%, while the lowest percentage was recorded after two weeks with percent of improvement 1152.33%. In contrast, ATP level showed significant decrease with time intersection, recorded percentage of improvement 60.40% after two weeks, where it still recorded significant decrease as compared to normal control rats. Also, AEC demonstrated the highest percentage of improvement 28.60 % after two weeks of treatment. Whereas, TA and inorganic phosphate recorded insignificant change with intersection of time. However, phosphate potential demonstrated significant increase 254.8% post two weeks of melatonin treatment as compared to normal control rats.

On the other hand, treatment of propagated-cirrhotic rats with melatonin after two weeks, showed significant ($p<0.05$) increase in AMP and ADP with percentages of improvement 126.5% and 406 %, respectively, while ATP, AEC and TA recorded an insignificant change as compared to normal control rats. Although, inorganic phosphate showed significant decrease with percentage of improvement 8.7%, whereas significant increase was observed in phosphate potential with percentage of improvement 208.7% as compared to normal control rats.

With respect to, cirrhotic-partially hepactomized rats treated with melatonin, significant increase in AMP and ADP levels was detected, since AMP showing its lowest value after two weeks with percentage of improvement reached to 4.37%. Also, ADP showed its highest value after two weeks with percentage of improvement 1069%. Whereas, ATP level showed significant decrease with the time intersection. In addition, AEC showed significant decrease after different durations. Whereas, TA showed significant ($p \leq 0.05$), increase after two weeks of melatonin treatment with percentage of improvement 62%. While, insignificant increase in TA level was detected after three days and one week. Moreover, inorganic phosphate demonstrated significant decrease in cirrhotic–partially hepatectomized rats treated with

melatonin with the time intersection as compared to normal control level with percentage of improvement 53.60% after two weeks. Moreover, phosphate potential demonstrated the highest percentage of improvement 64.90% after two weeks of treatment as compared to the normal control group.

In agreement with the present results, Okatani et al.[90] found that, melatonin has been reported to protect liver cells from damage after ischemia/reperfusion by restoring the respiratory control index, ADP/O, of State 3 respiration. Previous studies have demonstrated that cellular apoptosis consumes large amounts of nicotinamide adenine dinucleotide (NAD), and the process to resynthesize NAD, results in a decrease of cellular ATP levels[97]. Our data showed that ATP content was dramatically decreased in the lives of different experimental untreated models. These situations were correlated with elevated levels of oxidative stress and increased expression of pro-apoptotic genes[97]. In addition, Iida et al.[97]showed that, melatonin treatment was able to diminish the enhanced expression of pro-apoptotic genes like Bax, AIF or Bad and the activity of caspase-9. It could also prevent DNA fragmentation without influencing Bcl-2 expression, but improving the mitochondrial bioenergetic status in hepatic cells. Melatonin also reduced the release of cytochrome C into the cytosol, thus decreasing the activation of caspase-3 observed in rats submitted liver injuries. Moreover, melatonin-treated rats have been shown to display markedly fewer apoptotic (TUNEL positive) cells and less DNA fragmentation than did the untreated liver rat injuries[94]. Daily single injections of melatonin (50 mg/kg/day), has been shown to improve steato-hepatitis and to decrease apoptosis and cell injury in male rats submitted during four weeks to a methionine- and choline deficient diet[98]. Furthermore, treatment of ob/ob mice with 10 mg/kg/day melatonin for 12 weeks prevented the loss of mitochondrial respiratory chain activity, protected there complexes and subunits from degradation, and favored assembling of mitochondrial complexes[99]. Also, melatonin is a highly lipophilic molecule interacts with lipid bilayers and stabilizes mitochondrial inner membranes, an effect that may improve electron transport chain activity and thus increases ATP synthesis *via* these actions; melatonin preserves the integrity of the mitochondria and helps to maintain cell functions and survival[100, 101].

In a parallel study, Rodriguez-Reynoso[89] and Okatani et al.[90] reported that exogenous melatonin prevents alterations in the energy state of hepatocytes during ischemia/reperfusion in rat liver and attributed this effect to a reduced concentration of tumor necrosis factor-alpha and inhibition of nitric oxide synthase expression and nitric oxide production.

Concerning IL-1 and TNF-α, they are pro-inflammatory cytokines having multifunctional factors such as cytotoxic, cytostatic, immunomodulatory and other activities. TNF-α and IL-1 may contribute to cancer progression and metastasis[102]. In a parallel results with[103], found that, activation of pro-inflammatory TNF-α, IL-1ß, and IL-6 in a septic shock model as well as in thioacetamide intoxication[88, 104].

Treatment of hepatectomized rats with melatonin showed, percentages of enhancement amounting to 58.54, 34.163 and 20.13%, respectively for TGF-ß, IL-1 and TNF- α after two weeks treatment. Cirrhotic rats treated with melatonin showed significant increase in the level of TGF-ß, IL-1 and TNF-α with percentages of amelioration 60.98, 23.20 and 18.74%, respectively. In addition, cirrhotic hepactomized rats treated with melatonin, exhibited percentages of amelioration reached to 27.49, 28.64 and 34.31%, respectively for TGF-ß, IL-1 and TNF-α after two weeks of treatment.

In accordance with the present results, Wangetal.[23], Wang et al,.[69] and Sigala et al.,[105] reported that, melatonin protects immune liver injury and liver fibrosis by inhibiting inflammatory cytokines and oxidative stress. In addition, TNF-a and IL-1 in the DMN-induced liver injury rats were significantly regulated in melatonin- treated rats. Sigala et al.[105] added that DMN, induced oxidative stress and consequent lipid peroxidation exert harmful effects, as they associated with the pathogenesis of acute liver injury and melatonin is reported to exhibit a wide variety of biological effects, including antioxidant and anti-inflammatory. Thus, melatonin has a protective role in different types of liver injury and fibrosis. Moreover, Hu et al.,[79] found that, melatonin is capable of to reduce the severe extent of hepatic cell damage, inflammatory cells and TNF- α in alcoholic induced liver injury. Animal study shows that preventing lipid peroxidation with antioxidants is associated with reduced focal necrosis and inflammation[79, 106]. It is known that, melatonin has been found to possess higher antioxidant efficiency than vitamin E and GSH, as they extensively known as powerful antioxidants[31, 79]. Furthermore, melatonin decreased serum and tissue inflammatory cytokines levels, tissue lipid peroxidation, neutrophil infiltration and inhibited the apoptosis of hepatocytes. It was found that, Kupffer cells isolated from ethanol-fed mice produced high amounts of reactive oxygen species and tumor necrosis factor alpha, whereas Kupffer cells from melatonin treated mice produced less reactive oxygen species and tumor necrosis factor alpha compared with model alcohol-feeding mice. These findings suggested that melatonin may represent a novel, protective strategy against alcoholic liver injury by attenuating oxidative stress, inflammatory response and apoptosis[79].

Considering Epidermal growth factor (EGF), is a potent mitogen for hepatocytes and cholangiocytes and is thought to act as an immediate-early gene after partial hepatectomy. Since, regeneration is impaired in cirrhosis, we explored the expression of EGF in cirrhotic rat liver immediately after partial hepatectomy. The present study shows that EGF is increased in the cirrhotic liver. This

could be contributed to the increase activity in the remnant of normal parenchymal liver tissue observed in cirrhosis to compensate cirrhotic one**.** The lack of up-regulation after PH sheds doubt on the role of EGF as an immediate-early gene in hepatic regeneration[107]. In a parallel result Corpechotet al.,[108] showed that, cirrhosis is associated with a progressive increase in EGF expression. In addition, EGF and TGF-α, are also involved in the liver regeneration and are increased in cirrhotic livers. In contrast with the present results [108], recorded significant decrease in HGF in both regenerating and cirrhotic liver. This, observation may be due to the lack of parenchyma liver tissue. In this concern, and in concomitant with the present results, melatonin documented ability to regulate both the transcription of the receptor gene of estrogen (ER)[109-112], and the oncogenic potential of the growth hormone (GH) axis with prolactin-insulin-like growth factor-1 (IGF-1) and of GH-dependent growth factors, such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor (TGF), and hepatocyte growth factor (HGF), are aspects that certainly have an anticancer relevance[112, 113]. Thus, melatonin might be suggested as therapeutic modalities in experimental models of different liver injuries [114].

With regard to histopathological investigation, the present results showed in partial hepatectomized rats almost normal hepatocytes after one week and two weeks. These results are in agreement with the results of Gonzalez et al.[74]. While, propagated cirrhotic rats treated with melatonin for two weeks showed moderate fibrous tissue II in portal tract, moderate hydropic degeneration, moderate lymphocytic infiltration of portal tract. Propagated cirrhotic-partial hepatectomized treated after one week treatment with melatonin showing damage in the hepatic architecture and vacuolar degeneration while after two weeks treatment showed no fibrous tissue in portal tract, mild hydropic degeneration (red arrow), mild lymphocytic infiltration of portal tract an overall enhancement has been observed in all the pathological conditions this may be encountered to melatonin is highly effective in scavenging OH and other reactive oxygen and nitrogen species[31]**.** Melatonin inhibits free radical-mediated lipid peroxidation both *in vivo* and *in vitro*[92]**,** where oxidative stress or high phosphate concentrations; both these conditions are encountered during general liver injuries[90]. Also, this may be due to its wide range physiologic, antitumor effects, antiproliferative and possibly cytostatic effects[74].

CONCLUSION

Treatment with melatonin showed therapeutic effects through markedly accelerated liver regeneration after partial hepatectomy, attenuated DMN- induced liver injury by its ROS scavenging activities, stimulation of respiratory activity, modulated liver injury and liver fibrosis by inhibiting inflammatory cytokines and oxidative stress, stimulated hepatocyte mitosis, that consequently leads to the survival of rats with a high risk of postoperative liver failure after hepatectomy. These results ascertained scavenging free radicals and antioxidant activities of melatonin in enhancement regenerating liver after partial hepatectomy or DMN induced liver injury.

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