The Hepatoprotective Effect of Melatonin in male rabbits

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Abstract: Melatonin [N- acetyl- 5- methoxytryptamine] (ME), an endocrine product of pineal gland. The present work was conducted to investigate the hepatoprotective effect of exogenous (ME). Thirty six healthy male rabbits weighting 1500-1700g.were divided into six groups with 6 animals in each group. Animals in first group served as control, animals in second, third, fourth, fifth, and sixth groups were intraperitoneally (i.p) injected with D-Galactosamine (GalN) in a single daily dose of 50mg/kg period of 20 days for the induction of hepatocellular injury. The animals in third and fourth groups in addition to GalN were orally treated with ME in a single daily dose of 300μg/kg, as follows: animals in third group received ME at 9am; and animals in fourth group received ME at 9pm, period of 20 days. The animals in fifth and sixth groups, in addition to GalN, were orally treated with ME in a single daily dose of 600μg/kg, as follows: animals in fifth group received ME at 9am, and animals in sixth group received ME at 9pm period of 20 days. The level of Albumin, Total Protein, Alanintransferase (ALT), Asparatatetransferase (AST), and Alkalin-phosphatase (ALP) in serum was estimated. Results showed that ME significantly (P<0.01) reduced the toxicity of GalN.Results also showed that ME is more effective when it given at evening times.
Key words: Melatonin, Hepatoprotective effect, Antioxidant.

INTRODUCTION

Melatonin [N-acetyl-5-methoxytryptamine] (ME) is a secretory product of the vertebrate pineal gland [1]. Although melatonin was discovered to be a free radical scavenger just over a decade ago [2], the data documenting its ability to overcome oxidative stress has accumulated at a rapid pace and it is now abundant [3-7].

The efficacy of melatonin in functioning in this capacity relates to its direct free radical scavenging actions [5, 8], its ability to enhance the activities of a variety of antioxidative enzymes [9-11], its stimulatory actions on the synthesis of another important intracellular antioxidant, glutathione [12], its efficacy in reducing electron leakage from the mitochondrial electron transport chain [13], and its synergistic interactions with other antioxidants [14]. Moreover, in recent years it has become apparent that when melatonin scavenges radicals and related reactants, the products that are generated are also free radical scavengers thereby greatly exaggerating the antioxidant potential of melatonin [7]. Melatonin is a potent free radical scavenger, more than vitamin E which is the reference in the field [1]. Melatonin directly scavenges the highly toxic hydroxyl radical and other oxygen centered radicals and displays antioxidative properties: it increases the levels of several antioidative enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase [11, 12]. On the other hand; melatonin inhibits the pro-oxidative enzyme nitric synthase [15, 16]. Since considerable experimental evidence supports the idea that oxidative stress is a significant component of specific heart, blood vessels and CNS diseases, the ability of melatonin to protect against cardio- and neurodegeneration was tested in a multitude of models [17, 18]. At the present time, there is experimental evidence indicating that the quantity of melatonin endogenously produced, is relevant as a physiological antioxidant in normal conditions, whereas too experimentally evaluation the antioxidant activity of exogenous melatonin in mammals, the quantity of it should not exceed 1mg/kg to avoid the possible side effects [17, 19]. The administration of exogenous melatonin, should take into consideration that, antioxidant defense system displays a daily rhythm which is abolished by pinealectomy, or by light in mammals including man [20]. Few controlled trials studies showed that, in chronic hemodialysis patients, the oxidative stress induced by iron and erythropoietin given for treatment of anemia was prevented by oral administration of melatonin 0.3 mg/kg [21]. Preliminary results in septic newborns showed that high melatonin doses (20 mg per subject) significantly reduced serum levels of lipid peroxidation products and inflammation markers and increased the survival rate and improved the clinical outcome of patients [15]. Similarly,

increased blood levels of malondialdehyde and nitrite\nitrate observed in asphyxiated newborns were reduced by melatonin treatment (a total dose of 80 mg per infant). Three of the 10 asphyxiated newborns not given melatonin died within 72hrs. After birth, whereas none of the 10 who received melatonin died [22]. D-Galactosamine (GalN), an amino sugar, it was shown to cause liver damage, increase oxidative stress and increase of LPO products [23].

The goal of this study is to investigate the hepatoprotective role of exogenous ME; against GalN induced liver injury in male rabbits.

MATERIALS AND METHODS
Chemicals
D-Galactoseamine, white crystal powder, and Melatonin, white crystal powder, were manufactured by Sigma, St.Louis, MO, USA, and obtained from Faculty of Sciences, Cape town University, South Africa.

Experimental animals
A total number of 36 healthy male rabbits local breed weighted 1500-1700g.were used in the present study. Animals were randomly assigned to 6 groups as follows:

Group1: ($n = 6$) control animals, they received 10 ml.Saline once a day period of 20 days.

Group2: ($n = 6$) animals in this group were intraperitoneally (i.p.) injected with D-Galactosamine (GalN), in dose 50mg/kg./day, dissolved in saline, period of 20 days for the induction of hepatocellular injury.

Group3: ($n = 6$) animals in this group, were intraperitoneally (i.p.) injected with D- Galactosamine (GalN), in dose 50mg/kg./day, dissolved in saline, and orally treated with ME in dose 300µg/kg./day, dissolved in distilled water at 9am, for 20 days.

Group4: ($n = 6$) animals in this group, were intraperitoneally (i.p.) injected with D- Galactosamine (GalN), in dose 50mg/kg./day, dissolved in saline, and orally treated with ME in dose 300µg/kg./day, dissolved in distilled water at 9pm, for 20 days.

Group5: ($n = 6$) animals in this group, were intraperitoneally (i.p.) injected with D- Galactosamine (GalN), in dose 50mg/kg./day, dissolved in saline, and orally treated with ME in dose 600µg/kg./day, dissolved in distilled water at 9am, for 20 days.

Group6: ($n = 6$) animals in this group, were intraperitoneally (i.p.) injected with D- Galactosamine (GalN), in dose 50mg/kg./day, dissolved in saline, and orally treated with ME in dose 600µg/kg./day, dissolved in distilled water at 9pm, for 20 days.

All animals were maintained in standard environmental conditions; they were housed in glass house under normal light and dark cycle of day, and kept a standard commercial diet with water *ad libitum*.

All experiment was administrated in the Animal Physiology Laboratory, Department of Biology, Faculty of Science and Education, Amran University.

After 20 days the animals were fasted overnight for12hrs. Then they were sacrificed, the blood was immediately collected and centrifuged, and serum was discarded and kept at - 21 ° C for the biochemical testes.

Analysis

Alanine- aminotransferase (ALT) and Asparatate- aminotransferase (AST) Assay
The estimation was carried out according to the method originally developed by Reitman and Frankel [24].

Alkaline phosphatase Assay
ALP was determined using a colorimetric method as described by Kind and King [25].

Total Protein Assay
The total protein was determined by Biuret method explained by Tietz [26]

Albumin Assay
Serum albumin was determined according to the method of Doumas *et al*. [27].

STATISTICAL ANALYSIS
The statistical analysis was performed by SPSS; continuous data are expressed as mean ±S.E. Data were compared using one – way ANOVA. P value <0.05 was considered to be statistically significant.

RESULTS
Results in table1 show that the i.p. administration of GalN in dose 50mg/kg period of 20 days (group 2) resulted in high significant P<0.01 decrease in the level of albumin and total protein, the mean percent decrease in albumin and total protein was 67±0.87 and 70±2.12 respectively, as compared to control. GalN i.p. administration resulted also in high significant P<0.01 increase in the level of ALT, AST and ALP, in the mean percent 170±7.75, 180±6.09 and 183±8.34 respectively, as compared to control. The mean percent decrease in the level of albumin and total protein in the serum of animals treated with ME in dose 300µg/kg at 9am (group3) beside GalN, was 48±0.65 and 56±1.88 respectively, as compared to control, while the mean percent increase in the level of ALT, AST and ALP was 136±6.06, 155±5.87 and 144±6.73 respectively as compared to control. The mean percent decrease in the level of albumin and total protein in the

serum of animals treated with ME in dose 300µg/kg at 9pm (group4) beside GalN, was 35±0.85 and 39±1.08 respectively, as compared to control, while the mean

percent increase in the level of ALT, AST and ALP was 97±4.22, 127±6.05 and 100±6.87 respectively as compared to control.

Table-1: Level of studied parameters after 20 days of GalN and ME administration in dose 50mg/kg and 300µg/kg respectively

Parameters	Treatments Control	GalN	GalN+ ME at 9 am	GalN+ ME at 9 pm
Albumin g/dl	3.7±0.23	1.2±0.04	1.9±0.09	2.4±0.11
T.Protein g/dl	7.1±1.01	2.1±0.88	3.1±0.96	4.3±0.98
ALT U/L	41±4.93	111±8.76	97±6.77	81±8.44
AST U/L	36±3.44	101±9.73	92±8.65	82±6.44
ALP U/L	61±5.32	173±11.01	149±9.79	122±9.91

Values are mean of 10 animals ±S.E. $P < 0.01$ vs. control.

Obtained results in table2 show that, the mean percent decrease in the level of albumin and total protein in the serum of animals treated with ME in dose 600µg/kg at 9am (group5) beside GalN, was 21±0.17 and 28±1.54 respectively, as compared to control, while the mean percent increase in the level of ALT, AST and ALP was 92±6.34, 97±6.43 and 83±5.39 respectively as compared to control. The mean percent decrease in the level of albumin and total protein in the serum of animals treated with ME in dose 600µg/kg at 9pm (group6) beside GalN, was 10±0.66 and 14±1.98 respectively, as compared to control, while the mean percent increase in the level of ALT, AST and ALP was 29±6.18, 36±6.55 and 14±9.95 respectively as compared to control.

Table-2: Level of studied parameters after 20 days of GalN and ME administration in dose 50mg/kg and 600µg/kg respectively

Parameters	Treatments Control	GalN	GalN+ ME at 9 am	GalN+ ME at 9 pm
Albumin g/dl	3.7±0.23	1.2±0.04	2.9±0.88	3.3±0.91
T.Protein g/d	7.1±1.01	2.1±0.88	5.1±1.16	6.1±0.13
ALT U/L	36±3.44	101±9.73	70±6.56	49±7.12
ALP U/L	61±5.32	173±11.01	112±5.39	70±6.98

Values are mean of 10 animals ±S.E. $P < 0.01$ vs. control.

DISCUSSION
Our results clearly showed the hepatotoxicity of GalN. In agreement with previous study [23], the i.p. injection of GalN to rabbits in group 2 in dose 50mg/kg for 20 days resulted in high significant $p < 0.01$ increase in the level of ALT, AST, and ALP, and high significant $p < 0.01$ decrease in the level of albumin and total protein. The noticed increase in the levels of aminotransferases (ALT and AST) and the level of ALP as well as the decrease in the in the levels of total protein and albumin in the serum, are the major diagnostic symptoms of liver diseases [28]. Exposure to GalN leads to increase the oxidative stress and excessive of free radicals production, which attack many organic molecules in cells membrane including polyunsaturated fatty acid leading to increase in LPO and damage of cells and their function [27]. Several studies reported that reactive oxygen species (ROS) initiate LPO through the action of hydroxyl radicals [7, 29, 30].

Our results showed that, the toxicity of GalN was reduced in the treated with ME animals (groups 3, 4, 5 and 6), the protective effect of ME in our experiment was time of administration dependent effect. The high significant protective effect of ME was clear in the animals who received it at evening time (9pm), as compared to the animals who received it at morning time (9am). The protective effect of ME is relates to it antioxidant activity. Melatonin was shown to be effective in neutralizing a number of oxygen-based and nitrogen-based toxic agents, some of which are free radicals and some of which are related metabolites [5, 31]. ME was originally shown to detoxify the highly toxic hydroxyl radical ($^-$OH) [32]. Since this discovery, its scavenging repertoire has been expanded to include hydrogen peroxide (H_2O_2) [33], hypochlorous acid (HOCl) [34], single oxygen ($^1O_2$) [35], superoxide anion radical ($O_2\cdot-$), nitric oxide (NO·) [36, 37], peroxynitrite anion (ONOO$^-$) [4] and other free radicals [7].

The high significant efficacy of ME at the evening time is due to the well-known fact that light/dark cycle is the main regulating system of ME secretion, function and receptors in the body [38]. Light suppresses ME secretion and ME receptors activity, and vice versa, dark stimulates ME secretion and it receptors [39-40].

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