

## The Effect of Melatonin and/or Complex Vitamin B<sub>1</sub>,B<sub>6</sub>,B<sub>12</sub> in Modulating Epinephrine-induced Stress in Male Rats

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### ABSTRACT

*This study was undertaken to determine the modulating effects of intramuscular administration of melatonin (MT)(1mg/ kg) and/or Tri-B (B<sub>1</sub>, B<sub>6</sub> and B<sub>12</sub>) (20mg/kg) on body weight and some biochemical changes in rats induced by Epinephrine (Epi) injection. The data showed that MT and/or Tri-B treatment effectively improved the changes in malondialdehyde, lipid profile, blood sugar level and insulin. MT and/or Tri-B administration following Epi improved partially the decrease in body weight and liver glycogen levels. Tri B injection following Epi partially improved all the tested parameters except malondialdehyde and blood sugar level that completely improved in stressed rats. It was evident that a combination of MT and vitamin B complex had protective actions and further it was better than either of them introduced alone in stressed rats. The possible interaction between MT and Tri B provided further support to MT synergistic actions with the aim of advocating MT and Tri B as a possible synergistic therapy.*

**Key words:** Stress, Epinephrine, Melatonin, Tri-B, Rats

### INTRODUCTION

Stress is one of the basic factors in the etiology of a number of diseases. It can stimulate numerous pathways leading to an increased production of the oxidants adding to the oxidant burden associated with normal aerobic metabolism and its consequent damage to lipid, protein and DNA that appears to be a major contributor to aging and degenerative diseases of aging. Stress depletes important minerals, vitamins, and nutrients from the body. This deficit can have a significant impact on one's health, and leaving one vulnerable to certain acute and chronic health conditions (Maddock and Pariante 2001; Sabban and Kevetnansky 2001; Esch et al. 2002). Epinephrine (Epi) causes general physiological changes and plays a central role in the short-term stress reaction

that prepares the body for physical activity (fight or flight response) initiated by the sympathetic nervous system (Aronson 2000). Tachycardia, ventricular arrhythmias, hypertension, hyperglycemia, and even oxidative stress are side effects of this drug (Rakha et al. 2008). Hence, in the present study, Epinephrine was used to induce stress in the rats as an example of stressed animal. Melatonin is a lipophilic hormone, mainly produced and secreted at night by the pineal gland and is a powerful antioxidant with its high free radical scavenging activity (Barrenetxe et al. 2004). Melatonin is involved in many physiological functions such as immune response, energy metabolism and temperature regulation (Sahin et al. 2004). It has a neuroprotective effect against the cerebral ischemic injury, which may be attributed to its activity in preserving the oxidant-

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antioxidant status and electrolyte balance (Toklul et al. 2009). Vitamins B including B<sub>1</sub> (thiamine), B<sub>6</sub> (pyridoxine), and B<sub>12</sub> (cobalamin) are involved in many important physiological functions such as carbohydrates and fat metabolism, cell reproduction, and cell membrane permeability. Thiamine, or vitamin B<sub>1</sub>, has also shown anti-stress activity in the animals by protecting the cardiac tissue from stress-induced ischemia (Vinogradov et al. 1991). In addition, the antioxidative properties of vitamin B<sub>6</sub> have been discovered (Jain and Lim 2001; Matxain 2006). Based on human and animal research, B<sub>12</sub> may allow individuals to sustain an adaptive response and minimize some of the systemic effects of stress (Kelly 1999). In the present study, experiments were conducted in male rats to determine the effect of Epi and to promote the adoption of natural occurring supplements such as MT and/or vitamin B complex (B<sub>1</sub>, B<sub>6</sub> and B<sub>12</sub>) that might aid in the prevention and treatment of some harmful induced effects.

## MATERIALS AND METHODS

### Experimental animals

Adult male albino Spargue-Dawley rats (2.5-3 month old, 200-210 gm) were used as the experimental animals. Rats were environmentally adapted in the animal holding room for at least two weeks prior to the experiment. Animals were housed in groups in the stainless steel cages (four rats per cage) at room temperature (22-25°C), 60-70% relative humidity and a photoperiod of 12h/d. Food (pelleted rat chow) and drinking water were available *ad libitum*. The environment in which the rats were housed was built to be as free of contaminating trace element as possible. Rats were observed carefully during the acclimatization period to eliminate any animal showing the signs of disease, stress, physical damage or mortality.

### Chemicals

Epinephrine, Melatonin and Tri-B (vitamins B<sub>1</sub>, B<sub>6</sub> and B<sub>12</sub>) were purchased from Sigma Chemical Co. St Louis, (MO, USA).

### Experimental Design

One hundred forty male albino rats were divided randomly into seven groups (I-VII) (20 rats each):

**Group I** (control group): Animals of this group were injected with saline and served as control during the experimental period.

**Group II** (Epinephrine group): Animals of this group were injected with an intramuscular Epinephrine dose of 0.02 mg/kg according to Berg et al. (1996).

**Group III** (Melatonin group): Animals of this group were injected with an intramuscular MT dose of 1 mg/kg as described by Mamdouh et al. (1996) (negative control group).

**Group IV** (Tri-B group): Animals of this group were injected with an intramuscular Tri-B dose of 20 mg/kg according to Bolkent et al. (2008) (negative control group).

**Group V** (Epinephrine + MT group): Animals of this group were injected with an intramuscular dose of both Epinephrine and Melatonin using the same doses and manner as in the groups II and III.

**Group VI** (Epinephrine + Tri-B group): Animals of this group were injected with an intramuscular dose of both Epinephrine and Tri-B using the same doses and manner as in the groups II and IV.

**Group VII** (Epinephrine + Melatonin + Tri-B group): Animals of this group were injected intramuscularly with Epinephrine, Melatonin and Tri-B using the same doses and manner as in the groups II, III and IV.

The experimental period lasted for 10 consecutive days and the injections were carried out nearly at the end of light period/onset of dark period. Lights were off at the onset of the injections. Ten rats of each group were sacrificed after 3 h of receiving a single dose of saline, Epinephrine, Melatonin, and Tri-B as described above. Another 10 rats of each group were sacrificed after receiving the same doses daily for 10 consecutive days. In all the groups, the animals starved for 12 h prior to blood collection to ensure the homogeneous blood and organ samples. Blood was collected by decapitation of the animal after being lightly anaesthetized after 3 h of the injections on the 1<sup>st</sup> day and 10<sup>th</sup> day of the experiment.

### Body weight record

The body weight was recorded for each experimental animal by weighing the animal at the onset and the laps of the experimental durations.

### Preparation of serum

After 3 h of a single dose and 10 consecutive daily doses of Epi, MT, and Tri-B injections, rats were decapitated. The blood was allowed to clot in a

centrifuge tube and the serum was separated from the blood cells by centrifugation at 8000 rpm for 5 min. Quantitative measurement of the lipid peroxidation products malondialdehyde was determined according to the method of Janero (1990). The total lipid was estimated by the method of Zollner and Kirsch (1962). For the estimation of triglycerides (TG<sub>s</sub>) and free fatty acids (FFA<sub>s</sub>) the methods of Wahlefeld and Bergmeyer (1974) and Radwan (1978) were followed. Total cholesterol was determined by the method of Roeschlau et al. (1974) and high density lipoprotein (HDL) by Burstein et al. (1970). For the determination of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and blood sugar level the method of Trinder (1969) was followed. Insulin and liver glycogen were determined according to the methods of Sapin et al. (2001) and Hassid and Abraham (1963), respectively.

### Statistical analysis

**Table 1** - The effect of the intramuscular injection of Epi (0.02 mg/kg) with the intramuscular injection of the antidotes MT (1 mg/kg) and /or Tri-B (20 mg/kg) on the body weight (g), the concentration of MDA (μmol/L), triglycerides (mg/dl) and total free fatty acids (mM/μL) in the serum of male rats after 3 hrs of receiving a single dose and 10 daily consecutive doses.

Treated group	The body weight		MDA		Triglycerides		Total free fatty acids	
	3 hrs	10 days	3 hrs	10 days	3 hrs	10days	3 hrs	10days
Control	200.0 ± 5.60	215.0 ± 6.40	2.24 ± 0.23	2.25 ± 0.23	61.03 ± 4.35	61.00 ± 4.35	0.68 ± 0.030	0.67 ± 0.030
Epinephrine	180.0 ± 6.10 <sup>a</sup>	161.0 ± 7.00 <sup>a</sup>	3.35 ± 0.36 <sup>a</sup>	4.92 ± 0.40 <sup>a</sup>	125.0 ± 7.51 <sup>a</sup>	190.0 ± 8.66 <sup>a</sup>	1.20 ± 0.100 <sup>a</sup>	1.47 ± 0.120 <sup>a</sup>
Melatonin	200.0 ± 6.42	213.0 ± 8.30	2.15 ± 0.13	2.20 ± 0.12	53.00 ± 4.79	53.00 ± 4.31	0.60 ± 0.022	0.58 ± 0.020
Tri-B	200.0 ± 7.21	210.0 ± 8.00	2.20 ± 0.30	2.13 ± 0.20	64.00 ± 4.90	62.00 ± 5.43	0.65 ± 0.031	0.61 ± 0.037
Epinephrine + Melatonin	186.0 ± 6.82 <sup>a,b</sup>	189.0 ± 7.10 <sup>a,b</sup>	5.52 ± 0.28 <sup>b</sup>	2.72 ± 0.31 <sup>b</sup>	102.0 ± 6.89 <sup>b</sup>	62.00 ± 5.69 <sup>b</sup>	0.96 ± 0.091 <sup>b</sup>	0.70 ± 0.054 <sup>b</sup>
Epinephrine + Tri-B	184.0 ± 6.50 <sup>a,b</sup>	187.0 ± 7.00 <sup>a,b</sup>	3.13 ± 0.21 <sup>a,c</sup>	2.83 ± 0.33 <sup>a,c</sup>	110.0 ± 7.04 <sup>a,c</sup>	137.0 ± 7.31 <sup>a,c</sup>	1.01 ± 0.110 <sup>a,c</sup>	0.73 ± 0.091 <sup>a,c</sup>
Epinephrine + Melatonin +Tri-B	187.0 ± 6.00 <sup>a,b</sup>	193.0 ± 6.80 <sup>a,b</sup>	2.61 ± 0.23 <sup>b</sup>	2.70 ± 0.26 <sup>b</sup>	89.0 ± 5.86 <sup>b</sup>	79.00 ± 5.29 <sup>b</sup>	0.93 ± 0.085 <sup>b</sup>	0.72 ± 0.046 <sup>b</sup>

- Each value corresponds to a mean of 10 animals ± SEM.

-a,b and c are the statistically significant ( $P < 0.05$ ) when compared values of all experimental groups.

Results given in Tables 1 and 2 showed clearly significant elevations in triglycerides (TGs), free fatty acids (FFAs), total lipids, total cholesterol, low density lipoproteins in cholesterol (LDL), and very low density lipoproteins in cholesterol (VLDL) levels after Epi injection. On the other hand, high density lipoproteins in cholesterol

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS/ version 16) software. The significance of the differences between the groups was assessed by one-way ANOVA. All results were considered statistically significant at  $P < 0.05$ .

### RESULTS

The side effects of the intramuscular injection of Epi (0.02 mg/kg bwt) and the co-administration of Epi with MT(1mg/kg bwt) and / or Tri B (20 mg/kg bwt) on body weight and some biochemical parameters are shown in Tables 1 to 3. The intramuscular administration of Epi induced significant decrease in the body weight as compared to the control group (Table 1). The intramuscular injection of Epi induced significant elevations in malondialdehyde (MDA) concentration which was more pronounced after 10 consecutive daily doses as shown in (Table 1).

(HDL) was significantly decreased as compared to the control group. The intramuscular injection of Epi caused significant elevation in blood sugar level, while liver glycogen and insulin were decreased as compared to the control group (Table 3).

These results showed that co-administration of MT and/or Tri B following the Epi restored some of the weight loss since there were significant differences between these values and the values of control group. The MT and/or Tri B injection following the Epi completely improved the different changes in malondialdehyde (MDA)

concentration, lipid profile, blood sugar level and insulin as compared to both control and Epi groups. Also, the co-administration of MT and/or Tri B following the Epi partially improved the liver glycogen since there was significant difference between the value of this group and the value of the control group.

**Table 2** - The effect of the intramuscular injection of Epi (0.02 mg/kg) with the intramuscular injection of the antidotes MT (1 mg/kg) and /or Tri-B (20 mg/kg) on total lipids (mg/dl) , total cholesterol (mg/dl) , LDL (mg/dl), VLDL (mg/dl) , HDL (mg/dl) levels in the serum of male rats after 3 hrs of receiving a single dose and 10 daily consecutive doses.

Treated group	Total lipids		Total cholesterol		LDL		VLDL		HDL	
	3 hrs	10 days	3 hrs	10 days	3 hrs	10days	3 hrs	10days	3 hrs	10days
Control	539.5± 18.51	539.4± 18.51	78.00 ± 4.76	78.20 ± 4.76	25.50 ± 2.24	25.46 ± 2.24	12.20 ± 1.13	12.23 ± 1.13	40.50 ± 2.48	40.57 ± 2.48
Epinephrine	621.0± 36.39 <sup>a</sup>	696.0± 41.04 <sup>a</sup>	121.0 ± 6.58 <sup>a</sup>	141.0 ± 8.22 <sup>a</sup>	59.80 ± 4.10 <sup>a</sup>	74.00 ± 4.71 <sup>a</sup>	22.00 ± 2.21 <sup>a</sup>	38.00 ± 2.40 <sup>a</sup>	30.00 ± 3.55 <sup>a</sup>	29.00 ± 3.11 <sup>a</sup>
Melatonin	526.0± 21.55	519.0± 24.86	77.00 ± 4.12	75.80 ± 4.81	22.60 ± 2.12	20.30 ± 2.63	10.60 ± 0.95	10.60 ± 0.89	44.00 ± 2.21	43.90 ± 4.23
Tri-B	543.0± 26.77	546.0± 31.51	79.00 ± 5.36	80.00 ± 5.57	28.20 ± 2.94	23.00 ± 1.96	12.80 ± 1.17	13.20 ± 1.25	40.00 ± 3.06	43.00 ± 4.10
Epinephrine + Melatonin	606.0± 24.85 <sup>b</sup>	563.0± 37.70 <sup>b</sup>	110.00± 5.91 <sup>b</sup>	79.00 ± 5.61 <sup>b</sup>	51.40 ± 3.63 <sup>b</sup>	31.00 ± 2.81 <sup>b</sup>	20.40 ± 2.31 <sup>b</sup>	14.40 ± 1.73 <sup>b</sup>	39.00 ± 3.64 <sup>b</sup>	38.00 ± 3.71 <sup>b</sup>
Epinephrine + Tri-B	602.0± 27.5 <sup>a,c</sup>	671.0± 42.8 <sup>a,c</sup>	114.0 ± 7.29 <sup>a,c</sup>	107.0 ± 7.97 <sup>a,c</sup>	52.00 ± 3.11 <sup>a,c</sup>	42.10± 3.77 <sup>a,c</sup>	22.00 ± 1.90 <sup>a,c</sup>	21.40 ± 2.32 <sup>a,c</sup>	31.00 ± 3.37 <sup>a,c</sup>	33.00± 4.07 <sup>a,c</sup>
Epinephrine + Melatonin +Tri-B	596.0± 32.41 <sup>b</sup>	562.0± 40.57 <sup>b</sup>	96.0 ± 5.73 <sup>b</sup>	82.20 ± 6.13 <sup>b</sup>	54.20 ± 3.21 <sup>b</sup>	38.00 ± 2.81 <sup>b</sup>	19.80 ± 2.26 <sup>b</sup>	15.80 ± 0.93 <sup>b</sup>	42.00 ± 3.17 <sup>b</sup>	37.00 ± 3.81 <sup>b</sup>

- Each value corresponds to a mean of 10 animals ± SEM.

- a, b and c are the statistically significant ( $P < 0.05$ ) when compared values of all experimental groups .

**Table 3** - The effect of the intramuscular injection of Epi (0.02 mg/kg) with the intramuscular injection of the antidotes MT (1 mg/kg) and /or Tri-B (20 mg/kg) on insulin (mIU/ml), the blood sugar (mg/dl) levels in the serum of male rats and the liver glycogen content (mg/g ) after 3 hrs of receiving a single dose and 10 daily consecutive doses.

Treated group	Insulin		Blood sugar level		Liver glycogen content	
	3 hrs	10 days	3 hrs	10 days	3 hrs	10 days
Control	0.74 ± 0.031	0.75 ± 0.031	80.0 ± 4.21	81.0 ± 4.21	52.00 ± 2.21	52.10 ± 2.21
Epinephrine	0.33 ± 0.045 <sup>a</sup>	0.28 ± 0.055 <sup>a</sup>	132.0 ± 7.02 <sup>a</sup>	155.0 ± 7.61 <sup>a</sup>	39.0 ± 2.52 <sup>a</sup>	28.00 ± 3.33 <sup>a</sup>
Melatonin	0.79 ± 0.053	0.80 ± 0.084	82.0 ± 4.61	81.0 ± 4.06	49.0 ± 2.74	50.0 ± 3.14
Tri-B	0.71 ± 0.044	0.70 ± 0.053	81.0 ± 3.16	80.0 ± 5.51	48.0 ± 2.52	49.0 ± 2.68
Epinephrine + Melatonin	0.54 ± 0.066 <sup>b</sup>	0.61 ± 0.041 <sup>b</sup>	91.0 ± 5.65 <sup>b</sup>	80.0 ± 4.91 <sup>b</sup>	43.0 ± 2.65 <sup>a</sup>	31.00 ± 1.95 <sup>a</sup>
Epinephrine + Tri-B	0.36 ± 0.053 <sup>a,c</sup>	0.48 ± 0.046 <sup>a,c</sup>	94.0 ± 5.67 <sup>b</sup>	84.0 ± 3.31 <sup>b</sup>	40.0 ± 2.31 <sup>a</sup>	30.00 ± 2.01 <sup>a</sup>
Epinephrine + Melatonin +Tri-B	0.68 ± 0.047 <sup>b</sup>	0.69 ± 0.050 <sup>b</sup>	93.0 ± 6.7 <sup>b</sup>	81.0 ± 5.23 <sup>b</sup>	42.0 ± 2.72 <sup>a</sup>	33.00 ± 2.38 <sup>a</sup>

- Each value corresponds to a mean of 10 animals ± SEM.

- a, b and c are the statistically significant ( $P < 0.05$ ) when compared values of all experimental groups .

Partial improvement was observed after Tri B injection in the stressed rats since there were significant differences between the values of this group and the values of other experimental group in all the tested parameters, except malondialdehyde (MDA) concentration and blood sugar level, which were completely improved.

Rats treated with the MT or Tri-B (negative control groups) for 3 h and after 10 days showed no significant changes in the measured parameters (Tables 1-3).

## DISCUSSION

Experimental animals subjected to the Epi stress displayed a significant loss of body weight compared to their matched control in spite of free access to the food pellets and water. This could be due to a number of different potential mechanisms, which might be explained by the suppressed food appetite. Stress also might have hampered the utilization of food consumed during the stress period as previously reported by (Nagaraja et al. 2006). The present data were in agreement with Rybkin et al. (1997) exposing the rats to a single acute stress for 3 h of restraint, and Valle et al. (2000) and Harris et al. (2002) exposing the rats chronically to different stressors. Harris et al. (1998) demonstrated that exposing the adult rats to repeated restraint caused a temporary suppression of food intake and sustained reduction in body weight, compared with the non-restrained rats. Zhou et al. (2001) demonstrated that the rats exposed to restraint stress maintained a reduced body weight for extended periods of time during the post-stress period. The initial weight loss was associated with the hypophagia on the days of restraint and there was no evidence of compensatory over-eating during the post-stress period to make up for the stress-induced energy deficit.

Stressed rats administered with MT or Tri-B or both restored some of the weight loss by the Epi administration despite of not reaching the control body weight. Mosaad et al. (2005) demonstrated that melatonin was effective in the improving the food intake, body weight gain, serum total protein and albumin in the rats fed an ochratoxin A contaminated diet. Sankaran and Subramanian (2006) stated that the body weights of melatonin (0.5 mg and 1.0 mg/kg body weight) treated rats were increased significantly when compared to the

control rats. In contrast, Bénédicte et al. (2003) stated that melatonin decreased body weight gain and feed efficiency by half in Sprague Dawley rats fed with a high-fat diet.

Tri B utilized by the body during stress may be replaced by the introduction of exogenous Tri B, which are necessary for numerous body processes including metabolism. As indicated by the previous studies, a positive association with intakes of certain B vitamins and health was showed (Fletcher and Fairfield 2002; Anand 2005). The experimental induction of B<sub>6</sub> deficiency in a variety of animal species tends to produce delayed growth, reduced appetite, poor utilization of food, general weakness, and anemia (McDowell 2000). The administration of the vitamin B<sub>6</sub> for 15 days caused a significant increase in body weights in the diabetic rats (Bolkent et al. 2008).

The findings of this study further strengthened the previous studies reporting an increase in malondialdehyde (MDA) concentration after a single and repeated exposure to Epi stress compared to the unstressed control rats. MDA is sensitive marker for lipid peroxidation. An increase in free radicals causes overproduction of MDA. MDA level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients (Gaweł et al. 2004). Kowalczyk and Stryjecka-Zimmer (2002) showed that the levels of MDA were increased significantly in comparison to the level of control, in all different areas of the rabbit brain under oxidative stress.

The present study confirmed the role of melatonin as an antioxidant since it elicited an immediate effect on the concentration of MDA and a clear decrease was noted after 3 h of treatment. This decrease was more pronounced at the end of 10 days of repeated treatment in the stressed rats. It is known that the pineal gland has an antistressogenic role (Selma et al. 2006). MT administration prevented the changes in the MDA induced by Fe administration that generated the oxidative stress in chronic hemodialysis patients to treat anemia (José et al. 2001). MT caused a significant decrease in the brain, liver and kidney tissue MDA levels, which were increased because of streptozotocin-induced diabetes (Baydas et al. 2002). Sahin et al. (2004) found that the levels of MDA in serum, liver, heart and kidney was markedly increased in heat-stressed quail, and the levels significantly decreased by the MT

supplementation. Since MT readily crosses all the morpho-physiological barriers and easily penetrates in all the cells, wherever MT is located, either intracellularly and/or extracellularly, it can act merely by detoxifying a radical species *via* electron donation. This means that MT's action extends throughout the organism and to every cell (Reiter et al. 2007).

The supplementation of Tri-B vitamins significantly exerted a decrease in the MDA level after 10 days of repeated doses and this effect was more pronounced with the combination of the Tri-B vitamin with MT in the stressed rats after both the durations. B<sub>6</sub> acts as a cofactor and antioxidant (Chen and Xiong 2005). As previously mentioned that vitamin B<sub>6</sub> seemed to be associated with some defense mechanisms especially against lipid peroxidation in the tissues, this process occurred when the animals are totally lacked in the vitamin B<sub>6</sub> in the diet (Bordoni et al. 2006). Ullegaddi et al. (2006) stated that the supplementation with antioxidant vitamins and B-group vitamins separately or together caused a significant reduction in the plasma MDA concentration in the subjects in contrast to the increase seen in the control group. Kayali and Tarhan (2006) reported increased plasma and tissue lipid peroxidation has been reported in the rats receiving a vitamin B<sub>6</sub> deficient diet. Nachiket et al. (2010) showed that restraint stress (RS) enhanced the MDA levels in the serum and this was attenuated after pretreatment with vitamin B complex (100 and 200 mg/kg).

The results indicated that the administration of Epi provoked statistically significant increase in the concentration of TGs, FFAs, total lipids, total cholesterol, LDL, and in the VLDL after 3 h and 10 days of Epi injection; however, the HDL concentration was significantly decreased after 10 days of the injection. These prominent changes in lipid profile could be attributed to the increased fat catabolism since Epi activated the lipolysis in adipose tissue and increase of the FFAs flow to the liver, thus providing the liver with substrate for triacylglycerol synthesis and VLDL production, as a result increased TGs synthesis and secretion occurs (Sarav and Vlaykova 2005).

It has been demonstrated that stress induced increased concentration of the atherogenic LDL and decreased concentration of the antiatherogenic HDL in the monkeys (Kaplan et al. 1983) and rats (Spolsky and Mott 1987). Epinephrine infusion produced an increase in the plasma cholesterol

concentrations in the monkeys (Dimsdale et al. 1983) and rats (Kunihara and Oshima 1983). A majority of the studies have reported increases in the FFAs or serum cholesterol during either acute or chronic psychological stress also lipid fractions (LDL-C and HDL-C) are affected (Niaura et al. 1992). Mental stress influenced the lipid and lipoprotein concentrations, and an increase in total cholesterol, TGs and LDL cholesterol was generally observed (Muldoon et al. 1995). The low rate of epinephrine infusion significantly increased the lipolysis and plasma FFA mobilization (Ricardo and Edward 2000). Forced immobilization stress significantly increased the total cholesterol, LDL, VLDL (Sarvo and Valykova 2005) and TGs (Lata et al. 2004).

The administration of MT and/or Tri-B into the stressed rats completely improved the changes in TGs, FFAs, total lipids, total cholesterol, LDL, VLDL and HDL after 10 days of repeated treatment. The hypocholesterolemic effect of exogenous MT might work through the augmentation of endogenous cholesterol clearance mechanisms (Koppiseti et al. 2008). The anti-hyperlipidemic actions of MT has been studied by many researchers. Chan and Tang (1995) stated that MT exerted a beneficial effect by increasing the HDL/total LDL cholesterol ratio accompanied by the lowering of the cholesterol fraction associated with low density lipoproteins in secondary hypercholesterolemia. Sener et al. (2004) reported that the administration of MT caused significant increase in HDL-cholesterol with a clear significant drop in TGs, total cholesterol and LDL-cholesterol in the rats and mice fed hypercholesterolemic diet. Fabis et al. (2002) stated that single subcutaneous MT administration at a dose of 1 mg/kg body weight increased the concentration of HDL-cholesterol and decreased the level of FFAs in the blood of the rats. Paulis and Simko (2007) reported that MT had an extraordinary antilipidemic effect.

Lipid profile showed partial improvement in the Tri B treated rats after 10 days of treatment compared to the control group. It was hypothesized that B vitamins might enhance the catabolism of LDL by inhibiting their glycosylation or stimulation of hepatic cholesterol synthesis and decrease in the activity of the Krebs cycle (Hinse and Lupien 1981). Brattström et al. (1990) have previously observed that vitamin B<sub>6</sub> treatment reduced the plasma total cholesterol and LDL-cholesterol in atherosclerotic patients with

subnormal plasma B<sub>6</sub>. It has been reported that B<sub>6</sub> deficiency leads to fatty liver, accumulation of total lipids, mainly of TGs and cholesterol ester in the liver (Selvam and Ravichandran 1991) and impair the metabolism of polyunsaturated fatty acid (Tsuge et al. 2000). Improvement in the lipidemic profile in the dialysis patients were reported after supplementation with vitamin B<sub>6</sub> (Ziakka et al. 2001). Goksemin et al. (2006) reported that excessive vitamin B<sub>6</sub> administration with increasing treatment periods was likely to alter serum lipid levels. It has been reported that, vitamin B<sub>12</sub> deficiency entails disturbance in lipid metabolism. Kaya et al. (2009) stated that insulin resistance, obesity, and elevated homocysteine were associated with lower serum vitamin B<sub>12</sub> concentrations in the polycystic ovary syndrome patients.

The intramuscular injection of the Epi in the present study was associated with hyperglycemia confirming other investigations of the hyperglycemic effect of catecholamines (CATs) (Zardoz et al. 2006). This elevation could be due to increased gluconeogenesis and glycogenolysis (Nirupama et al. 2010) insulin resistance (Farias-Silva et al. 2002). Also, under stressful states, epinephrine depresses insulin secretion and activates beta receptors of pancreatic alpha cells, and stimulates glucagon production (Hadley and Levine 2007). Restraint for 1h (Zardoz et al. 2006), water immersion (Radahmadi et al. 2006) in the rats, and water spray bath for 15 minutes in the cats (Rand et al. 2002) resulted in increased serum glucose levels.

In the present study, elevated serum glucose was decreased with dietary melatonin supplementation. Similar effects of different antioxidants on the glucose and lipid metabolism have been reported (Sahin et al. 2002). The effect of MT on glucose level may be explained by modification of insulin secretion and/or change of cell sensitivity to insulin. MT is assumed also to act directly on the target cells (hepatocytes and pancreatic  $\beta$ -cells containing MT-binding elements) (Peschke et al. 2000). Studies have shown that MT influences glucose homeostasis (Reis et al. 1996) and resulted in the modification of carbohydrate metabolism in the rats (Markova et al. 2004). MT administration to pinealectomized animals adjusted the average daily glucose concentration (La Fleur et al. 2001). There is evidence that MT reduction can be involved in the genesis of diabetes mellitus type 2 since diabetics may present abnormally low levels

of this hormone (Peschke et al. 2006). Melatonin might be considered as a factor regulating glucose metabolism by affecting glucose-metabolizing enzyme activities, restoring tissue redox-balance and nitric oxide bioavailability (Elena et al. 2007). The administration of B vitamins completely abolished the glycemic effect of the Epi after 10 days of the injection. There is some evidence that supplemental B<sub>1</sub> may, in some cases, help correct the abnormality (Bakker et al. 1998). In vitro studies have shown that pyridoxamine can inhibit the formation of glycation end products (Khalifah et al. 1999). Vitamin B<sub>6</sub> supplementation restores normal glucose tolerance (Jain 2007). Supplementation with B<sub>6</sub> has also been shown to lower the blood glucose levels in streptozotocin-diabetic animals (Bolkent et al. 2008).

A significant decrease in liver glycogen content was reported after the two experimental durations of the Epi injection. Epi is known to stimulate the glycogenolysis using the enzyme glycogen phosphorylase (Richter et al. 1982). Epi also inhibits the glycogenesis (Wolfe et al. 1991). These results were also in line with that obtained by Goda et al. (1991) who mentioned that prolonged immobilization through the suspension of the rats caused appreciable alterations in hepatic glycogen content with the elevated glucose-6-phosphatase activity. Also, Epinephrine promotes glycogen breakdown within the muscle, stimulates glucose release from the liver, decreases plasma insulin and stimulates both glucose uptake and oxygen uptake (Bonen and Homonko 1994; James et al. 1999).

The administration of MT and/or Tri-B following the Epi could not improve the decrease in the hepatic content of glycogen in the two previously mentioned experimental times. However, melatonin has been found to elevate the liver and muscle glycogen contents in non-exercised rats and to spare the glycogen stores in the liver and muscle of exercised rats through the changes in carbohydrate and lipid utilization (Mazepa et al. 2000; Sánchez-Campos et al. 2001). Vitamin B<sub>12</sub> could suppress the decrease in the hepatic glycogen content in the rats exposed to noise stress (Zhu et al. 2006).

Based on the present findings, it could be concluded that MT could be effective in preventing the diseases related to lipid disturbances and promote clinical trials using melatonin to develop a plan for their treatment. Also, the combination of MT and Tri B could be

more effective than each one alone in the treatment of side effects of oxidative stress.

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