UNIVERSITI TEKNOLOGI MARA

LEPTIN INDUCED CHANGES IN SPERM PARAMETERS IN SPRAGUE-DAWLEY RATS: ROLE OF OXIDATIVE STRESS AND ITS PREVENTION BY MELATONIN

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a panel of examiners has met on 20th January 2016 to conduct the final examination of Fayez A. M. Almabhouh on his Doctor of Philosophy thesis entitled " Leptin Induced Changes in Sperm Parameters in Sprague-Dawley Rats: Role of Oxidative Stress and Its Prevention by Melatonin" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examniners recommends that the student be awarded the relevant degree. The panel of Examiners was as follows:

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AUTHOR'S DECLARATION

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ABSTRACT

Exogenous leptin administration has been shown to adversely effect sperm count and sperm morphology in normal adult rats. It is however unknown if it affects sperm *DNA* integrity and increases apoptosis of sperm and testicular tissue cells. This study therefore investigated the effect of leptin administration on sperm count, morphology, sperm *DNA* integrity, sperm *DNA* damage, apoptosis and histone-to-protamine ratios and its prevention by melatonin in Sprague-Dawley rats. It also examined the reversal of these effects on sperm parameters for up to 56 days following cessation of leptin treatment. Seventy-eight male Sprague-Dawley rats, aged 12 weeks, were randomized into 13 groups, Group 1 rats acted as a control and were given saline. Group 2 rats were given 60 μg/kg body weight daily of leptin for 42 days. Group 3 (leptin-melatonin-10) rats were given 60 μg/kg/day of leptin and 10 mg of melatonin/day/kg body weight in drinking water. Group 4 (leptin-melatonin-20) rats were given 60 μg/kg/day of leptin and 20 mg of melatonin/day/kg body weight in drinking water. Group 5 (melatonin-10) rats were given 10 mg of melatonin/day/kg body weight in drinking water. The remaining 8 groups were divided into four leptin and four saline treated control groups to examine the reversal of adverse effects of leptin. They were given intra-peritoneal (i.p.) injections of leptin daily at a dose of 60 μg/kg body weight for 42 days. Control rats received 0.1 ml of 0.9% saline. On day 43, one group of leptin treated rats and one group of age-matched saline treated control rats were euthanized for collection of epididymal sperm. The remaining three groups together with their age-matched saline treated controls were allowed to recover further for either 21, 42 or 56 days. Sperm count, morphology, histone-to-protamine ratios, 8-OHdG, apoptosis, sperm *DNA* damage, and gene expression profiles using micro-array analysis of the rat testes were determined. Data were analyzed using ANOVA and post-hoc analysis and presented as mean ± SEM. Compared to the controls, sperm count was significantly lower whereas the fraction of sperm with abnormal morphology, histone-to-protamine ratios, the level of 8-OHdG, apoptotic activity and sperm *DNA* fragmentation were significantly higher in leptin treated rats but not in leptin-melatonin-20 rats. Micro-array analysis revealed significant up-regulation of the expression of respiratory chain enzymes, apoptosis, *DNA* damage genes and down-regulation of anti-oxidant enzyme genes. All these differences were still evident at days 21 and 42 but not at day 56 of recovery period. In summary, it appears that leptin administration significantly decreases sperm count and down-regulated the anti-oxidant enzyme genes. It increases the fraction of sperm with abnormal morphology, *DNA* damage, apoptosis, *DNA* fragmentation and expression of caspase-independent apoptosis genes and *DNA* damage marker genes. These effects are prevented by concurrent administration of melatonin at dose of 20mg/kg. It appears also these adverse effects of leptin on sperm parameters are completely reversed within 56 days post-treatment in Sprague-Dawley rats.

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