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ROLE OF PI3K IN LEPTIN INDUCED ADVERSE EFFECTS ON SPERMATOZOA AND TESTICULAR TISSUE IN MALE SPRAGUE DAWLEY RATS

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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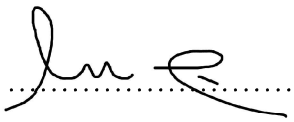
Signature of Student	:	
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ABSTRACT

Exogenous leptin administration exerts adverse effects on sperm count and sperm morphology in rats. However, the exact mechanism for this is still unknown. The PI3K signalling pathway is among the probable pathways through which these effects might be mediated. This study therefore examines the effects of PI3K pathway inhibitor, LY294002, on sperm parameters and testicular tissue in rats. Male Sprague-Dawley (SD) rats, aged 16 weeks, were given intraperitoneally either 0.1 ml of normal saline (Control), or 60 µg/kg of leptin (Leptin), or 60 µg/kg of leptin + 1.2 mg/kg of PI3K inhibitor, LY294002 (Leptin-I) for two weeks. Body weight and food intake were recorded at days 1 and 14. Animals were euthanized at day 15. The epididymides and testes were excised and weighed. Total sperm count and percentage of sperm with abnormal morphology were determined. Testosterone, inhibin B, 8-OHdG and Akt-ratio were determined using ELISA. Seminiferous tubule epithelial height (STEH) and diameter (STD) were examined using H&E staining method. Junctional proteins (JP), Cx43 and Occludin were examined using immunohistochemistry (IHC). All data were analysed using ANOVA. There were no significant differences in body weight, organ weight, or food intake between Leptin group and Control group over the 2 weeks. Body weight was significantly lower while epididymal weight was significantly higher in Leptin-I group when compared to Leptin group ($P<0.05$ respectively). No significant differences were found in their food intake and testicular weight. Sperm count was lower and fraction of sperm with abnormal morphology was higher in the Leptin group compared to controls ($P<0.05$, $P<0.01$). Sperm count was significantly higher in Leptin-I group when compared to the Control group ($P<0.01$) and Leptin group ($P<0.001$). The fraction of sperm with abnormal morphology was significantly lower in Leptin-I group when compared to the Leptin group ($P<0.001$). Testosterone levels in the Leptin-I group of rats was significantly lower compared to that in Leptin group ($P<0.05$), but no significant difference was seen in inhibin B levels between all three groups. The 8-OHdG levels in testes and sperm DNA was significantly higher in Leptin group when compared to Control group ($P<0.001$ in testis; $P<0.01$ in sperm). The 8-OHdG levels in Leptin-I group was significantly lower than that of the Leptin group ($P<0.001$) in the testis, but not in spermatozoa. STEH was significantly lower in Leptin Group compared to Control ($P<0.01$), but not STD. STEH and STD were significantly higher in Leptin-I group when compared to Leptin group ($P<0.001$, $P<0.01$ respectively). Expression of Cx43 was significantly higher in Leptin group compared to Control group ($P<0.05$) but not Occludin. Expression of Cx43 in Leptin-I group was significantly lower compared to that of Leptin group ($P<0.05$). Although not statistically significant, ratio of phosphorylated Akt to total Akt appeared to be slightly higher in Leptin group compared to Control group, but slightly lower in Leptin-I group compared to Leptin group. In conclusion, the adverse effects of leptin on sperm and testicular tissue, such as lower sperm count and higher percentage of sperm with abnormal morphology, increased oxidative damage in testis and sperm, deterioration of testicular tissue, disruption of the integrity of the BTB were all prevented by the PI3K pathway inhibitor, LY294002 when administered via the intraperitoneal route at a dose of 1.2 mg/kg/day for 14 days. This suggests the potential role of the PI3K signalling pathway in mediating the adverse effects of leptin on sperm and testicular tissue.

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