# PROFORTIL PREVENTS SOME OF THE LEPTIN-INDUCED ADVERSE EFFECTS ON SPERM IN SPRAGUE-DAWLEY RATS

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## INTRODUCTION

Leptin, a cytokine hormone, is primarily produced by the white adipose tissue (*Singh 2009*) and plays an important role in numerous systems of the body. In obesity, as the amount of adipose tissue increases, so do the levels of leptin. Excessive levels of leptin can have a detrimental effect in the body. This negative effect is also seen in the male reproductive system. Studies have shown that administration of exogenous leptin to Sprague-Dawley rats results in an increase in the fraction of sperm with abnormal morphology, levels of 8-OHdG and sperm DNA fragmentation and a decrease in total sperm count (*Haron et al. 2010*; *Almabhouh et al. 2014; Almabhouh et al. 2017; Mokhtar et al. 2018*). Leptin has been shown to increase in the levels of reactive oxygen species in the male reproductive system (*Abbasihormozi et al. 2012*). The data suggest that the negative effects of leptin involve oxidative stress. Indeed, when melatonin, a potent antioxidant, was administered to leptin-treated rats, it prevented the negative effects of leptin (*Almabhouh et al. 2017*). Profortil® is a health supplement composed of different antioxidants (L-carnitine, L-arginine, Coenzyme Q10, vitamin E, glutathione, zinc, selenium and folic acid). It is used clinically in the treatment of male infertility. This study, therefore, investigated the effects of Profortil® on leptin-induced adverse effects on sperm parameters in Sprague-Dawley rats.

## METHODOLOGY

4 groups of male Sprague Dawley rats, 11–14 weeks old (300–400 g)

- Control: 0.9% Normal Saline (N/S) 0.1 ml, *i.p.* (n=6)
- Leptin 60µg/kg/day, *i.p.* (n=6)
- Profortil 50 mg/kg/day, + 0.9% N/S 0.1 ml, oral (n=6)
- Leptin 60 µg/kg/day, *i.p.* + Profortil 50mg/kg/day, oral (n=6)

Treatment was given once daily. Profortil was given for 3 weeks. Leptin was given for 2 weeks. Treatments were given concurrently. Body weight and food intake were measured once a week.

At the end of the treatment, the animals were lightly anaesthetized with diethyl ether and euthanized by cervical dislocation.

Testes and cauda epididymides were collected and weighed. The cauda were used for analysing total sperm count and morphology of sperm. Testes were stored at -80ºC for further analysis.

Total sperm count and sperm with abnormal morphology was observed using Makler Chamber

Concentration of testosterone and the enzymes involved in its synthesis (CYP17a1, CYP19a1 and 17βHSD) were measured in the testicular tissue by ELISA

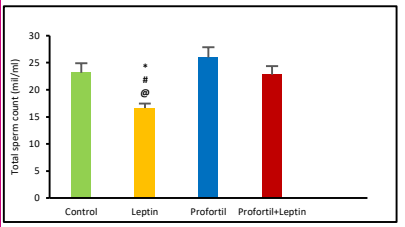
Concentration of 8-OHdG was measured in the testicular tissue by ELISA

Total antioxidant capacity (TAC) was measured in the testicular tissue by a TAC assay kit

Data were analysed using one-way ANOVA followed by Tukey's post-hoc analysis. Results were considered significant at $p < 0.05$.

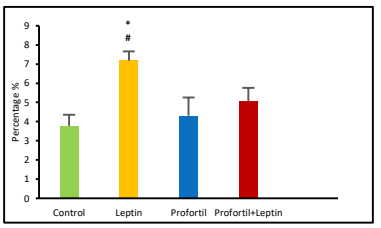
## RESULTS

### 1. TOTAL SPERM COUNT



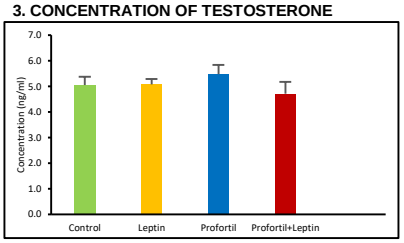

Total sperm count was significantly lower in the leptin group compared to the other three groups. No significant difference was observed in between the control, Profortil and Profortil+leptin groups.

### 2. SPERM WITH ABNORMAL MORPHOLOGY

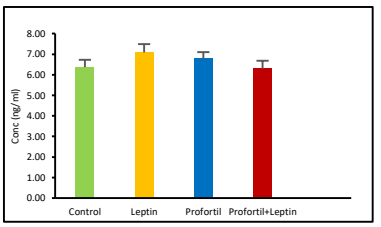



Percentage of sperm with abnormal morphology were significantly higher in the leptin group compared to the control and Profortil group. No significant difference was observed between leptin and Profortil+leptin groups.

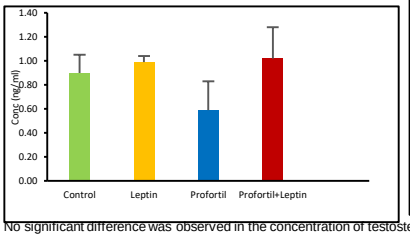
### 3. CONCENTRATION OF TESTOSTERONE



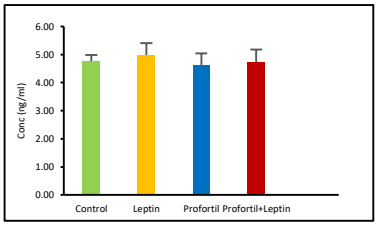

### 4. CONCENTRATION OF CYP17A1




### 5. CONCENTRATION OF CYP19A1

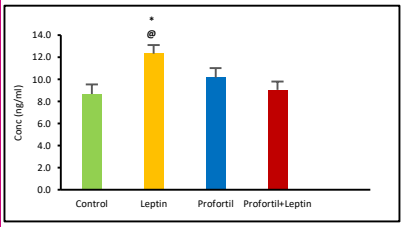



### 6. CONCENTRATION OF 17βHSD



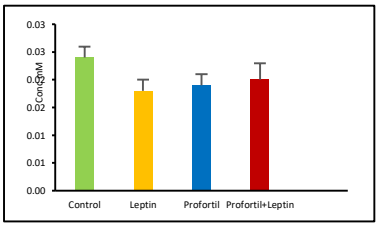

No significant difference was observed in the concentration of testosterone, CYP17a1, CYP19a1 and 17βHSD between the groups.

### 7. CONCENTRATION OF 8-OHdG




Concentration of 8-OHdG was significantly higher in the leptin treated rats compared to that in the control and Profortil+leptin treated rats. No significant difference was observed between the leptin and Profortil only treated rats.

### 8. TOTAL ANTIOXIDANT CAPACITY




No significant difference was observed in the total antioxidant capacity between all the groups.

### 9. BODY WEIGHT

| | Week 0 | Week 2 |
|---|---|---|
| Control | 346.33 ± 20.04 g | 377.67 ± 20.81 g |
| Leptin | 326.50 ± 10.13 g | 359.13 ± 11.60 g |
| Profortil | 392.50 ± 12.97 g | 404.00 ± 16.70 g |
| Profortil + Leptin | 383.71 ± 10.99 g | 401.14 ± 19.14 g |

### 10. FOOD INTAKE

| | Week 0 | Week 2 |
|---|---|---|
| Control | 21.33 ± 0.80 g | 21.15 ± 0.79 g |
| Leptin | 20.67 ± 0.94 g | 19.88 ± 0.67 g |
| Profortil | 20.17 ± 1.04 g | 20.00 ± 1.51 g |
| Profortil + Leptin | 19.14 ± 0.89 g | 19.86 ± 2.14 g |

### 11. ORGAN WEIGHT

| | Right Testis (g) | Left Testis (g) | Right Cauda (g) | Left Cauda (g) |
|---|---|---|---|---|
| Control | 1.53 ± 0.08 | 1.53 ± 0.07 | 0.24 ± 0.01 | 0.23 ± 0.01 |
| Leptin | 1.47 ± 0.02 | 1.46 ± 0.02 | 0.24 ± 0.01 | 0.23 ± 0.01 |
| Profortil | 1.60 ± 0.05 | 1.60 ± 0.06 | 0.25 ± 0.02 | 0.26 ± 0.01 |
| Profortil + Leptin | 1.61 ± 0.05 | 1.60 ± 0.05 | 0.26 ± 0.01 | 0.27 ± 0.01 |

No significant differences was present between either the body weight or food intake or organ weights of rats in all the groups.

**All data are expressed as mean ± SEM.**
*** p < 0.05 compared to control, # p < 0.05 compared to Profortil, @ p < 0.05 compared to Profortil + leptin**

## DISCUSSION & CONCLUSION

Results of the present study show that Profortil® when given concurrently with leptin to rats may prevent the adverse effects of leptin on total sperm count but not on sperm morphology. This is evidenced by the significantly higher sperm count seen in rats treated with Profortil®+leptin compared to that in the leptin only treated group, but no change was observed in the percentage of sperm with abnormal morphology in these rats. No significant differences were observed in the concentration of testosterone, and the enzymes involved in its synthesis, namely CYP17a1, CYP19a1 and 17βHSD, in all the groups. This finding seems to indicate that exogenous leptin treatment does not interfere in testosterone activity. However, as sperm count and sperm morphology are negatively impacted by leptin, this means that leptin is mediating its effects through some other means. One possible mechanism may be via oxidative stress, as indicated by the high levels of 8-OHdG in leptin-treated rats. 8-OHdG is a biomarker specific for oxidative stress-induced sperm DNA damage. Treatment of leptin-induced rats with Profortil® showed that the antioxidants present in this concoction were able to prevent the deleterious effects of oxidative stress caused by leptin.

In conclusion, it seems that concurrent administration of 50 mg/Kg of Profortil® to rats prevents the leptin-induced decrease in total sperm count and increase in 8-OHdG levels. Perhaps a higher dose, or longer duration of treatment may lead to more significant results.

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