Novel vitamin D-Nanoemulsion improves testicular function of vitamin D deficient rats

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Abstract

Background and aim Vitamin D (VD) deficiency is associated with disturbed reproductive functions in male rats which might be ameliorated by vitamin D (VD) supplementation. In this study, the efficacy of a novel VD carrier, developed by sonication and pH-shifting of pea protein isolate (nanoemulsion), in improving testicular function of VD-deficient rats was investigated.

Methods Thirty weaned male albino rats (92.62±13.61g) were divided into 2 groups. Group I (n=10) was fed a synthetic AIN-93G diet while Group II (n=20) was fed a customized AIN-93G diet with carbohydrate 59.3%, protein 18.1%, fat 7.1%, fiber 4.8%, VD (0–50 IU/kg) and calcium (5.1 g/kg) for 6 weeks. Hypovitaminosis D was confirmed by taking blood samples from the lateral tail vein. Rats in group I were used as a sufficient control (SC group), while those of group II were further subdivided into two groups (n=10 each): a nanoemulsion without VD deficient control (DC group) and a nanoemulsion with VD treatment (DT group). Animals were treated for 2 weeks with a gavage dose (1 mL of 27 μg/mL) every other day. Body weights were measured daily. Plasma 25-hydroxy vitamin D (25OHVD) and testosterone (T) were measured by ELISA. After euthanasia, testis were isolated, sectioned, stained and examined under light microscope. Image J was used to analyze the selected regions of interest (ROIs).

Results VD deficient diets resulted in low serum 25OHVD levels in SC vs. deficient groups (36.8±9.1 vs. 15.9±5.8 μg/mL, p<0.001), but no changes in body weight. VD deficient rats showed marked reduction of T (DC vs. SC, 0.83±0.22 vs. 3.06±0.74 ng/mL, p<0.001). Serum 25OHVD was not significantly correlated with T levels (r=0.408, p=0.18).

Histology showed a significant reduction in the Leydig cell mass and degenerative changes in all levels of the spermatogenesis (figure a and b). After treatment, DT group showed 6-fold increase in serum T in comparison with that of DC group and double that of SC (7.30±1.18 vs. 0.83±0.22 and 3.06±0.74 ng/mL respectively, p<0.001 for all).

Microscopically, selected ROIs showed marked improvement of germinal epithelium and all stages of spermatogenesis, together with an increase in Leydig cell count.

Conclusions Oral VD supplementation using pea protein nanoemulsion could improve serum T levels and ameliorate VD deficiency-induced histopathological changes in testis. Future research should investigate the underlying mechanisms.

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Testicular sections in vitamin D sufficient (a) and deficient D deficient (b) before treatment after 6 weeks of VD deficient diet.

Testicular sections in vehicle treated (DC group) (c) and VD+nanoemulsion treated (DT group) (d) after 2 weeks treatment period.