The present study aimed to investigate the effects of fortification and nano-size reduction on absorption and bioavailability of calcium from the fortified milk in ovariectomized (OVX) and OVX-osteoporosis rats. The main goal was to determine the effect of daily consumption of experimental diets on serum calcium, feces calcium, bone calcium, bone breaking strength and bone morphology among rats. This study was conducted to optimize the preparation of nano-fortified milk powder. Then, the calcium bioavailability in 64 female OVX rats and OVX-osteoporosis rats was investigated. The results revealed that bone breaking strength (maximum load) and bone calcium increased with consumption of nano-fortified diet compared with fortified diet. The same observations were seen for bone morphology. OVX rats exhibited higher calcium bioavailability and absorption than OVX-osteoporosis rats with regard to fortified and nano-fortified diets. The amount of calcium absorption from fortified and nano-fortified milk powders in OVX rats was 63.54% and 89.06%, respectively. The bioavailability of calcium from fortified and nano-fortified milk powders in OVX rats was 24.64% and 41.65%, respectively. Moreover, the calcium bioavailability (%) from fortified and nano-fortified milk powders in OVX-osteoporosis rats was 9.74% and 30.17%, respectively. In OVX-osteoporosis group, the absorption levels of calcium from the same experimental diets were 60.54% and 89.09%, respectively.