RESULTS

Aromatase inhibition *in vivo* has been studied by measuring the inhibition of the androstenedione-induced uterine hypertrophy in immature female rats. The results showed that letrozole (10 μg/kg) could significantly inhibit uterine hypertrophy in positive control group that received androstenedione 30 mg/kg/day. Furthermore, quinoline derivative could decrease the androstenedione-induced uterine hypertrophy in a dose-dependent manner. Interestingly, there was no significant difference between inhibitory potency of letrozole and quinoline derivative [Figure 3]. Letrozole inhibits the androstenedione-induced uterine hypertrophy in immature female rats with an IC_{50} of 1.5 μg/kg, and IC_{50} of quinoline derivative was 4.1 μg/kg as shown in Figure 4.

Effects of single oral doses of quinoline derivatives and letrozole on serum concentrations of cortisol and aldosterone as an *in vivo* aromatase selectivity assay are shown in Figure 5. Male rats received a subcutaneous injection of 10 mg/kg of ACTH, and 16 h later, they were treated with 4 mg/kg of either compound 8b or letrozole (control group received normal saline). After 2 h, all animals were beheaded, and their whole blood was collected. Serum concentration of aldosterone and cortisol was measured successful by ELISA assay. The results showed that high dose of letrozole could significantly decrease the serum concentration of aldosterone and cortisol as compared to control group.

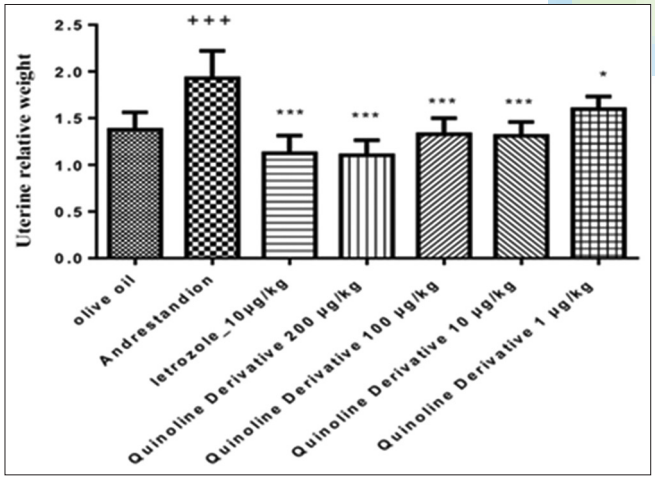

Figure 3: Inhibition of the androstenedione-induced uterine hypertrophy by quinoline derivatives and letrozole as an assay for the inhibition of aromatase *in vivo*. The immature female rats were treated with different doses of either quinoline derivatives or letrozole orally as well as IP injection of standard dose of androstenedione (30 mg/kg/day) for 4 consecutive days. Negative control group has been orally treated with olive oil, and positive control has received androstenedione. Results were expressed as uterine relative weight as a ratio of (uterine wet weight [mg]/body weight [g]) treated sample/(uterine wet weight [mg]/body weight [g]) negative control. The values are shown as mean ± standard error of the mean of determinations carried out of six rats in each group. +++$P \leq 0.001$ to negative control and ***$P \leq 0.001$ to positive control

DISCUSSION

Nowadays, aromatase competitive inhibitors are the first choice as adjuvant therapeutics for postmenopausal breast cancer patients. Researchers are interested in developing the new nonsteroidal aromatase competitive inhibitors with higher specificity and potency and lower side effects. The chemical structure of nonsteroidal AIs consists of two parts. One part is the azole part having nitrogen atom which binds to the heme iron atom of aromatase. The second part is usually the bulky aryl part that is hydrophobic and acts the same as the steroid ring of the substrate.[13] Ghodsi *et al.* designed and synthesized the quinoline derivatives (8a-g) as nonsteroidal AIs, and *in vitro* biological evaluation was also studied.[15] The ability of the quinoline derivatives 8a-g to inhibit aromatase enzyme activity was determined in subconfluent H295R cells by the titrated water release assay using [1-3H (N)]-androst-4-ene-3,17-dione, and letrozole (potent AI) at 5 μM concentration was used as positive control according to the previously reported method. Unexpectedly, most of the compounds were not able to inhibit aromatase enzyme activity at concentration ≤10 μM (in comparison with 5 μM letrozole). However, the compounds 8a, 8b, and 8c in this series display inhibition of aromatase enzyme activity at concentration ≤10 μM and 8b inhibited the enzyme activity strongly and was more potent than the reference drug letrozole. These results indicated that some of the lipophilic compounds, such as 8a, 8b, and 8c, apparently penetrate cells and inhibit aromatase activity. The significant inhibition of aromatase activity by compound 8b may be

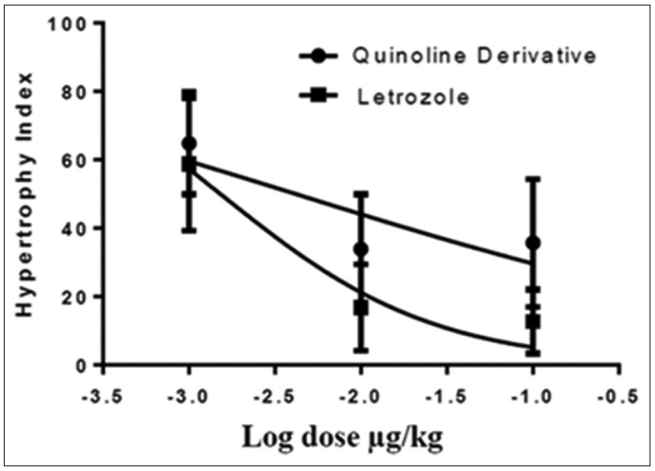

Figure 4: Uterine hypertrophy index of letrozole and quinoline derivative

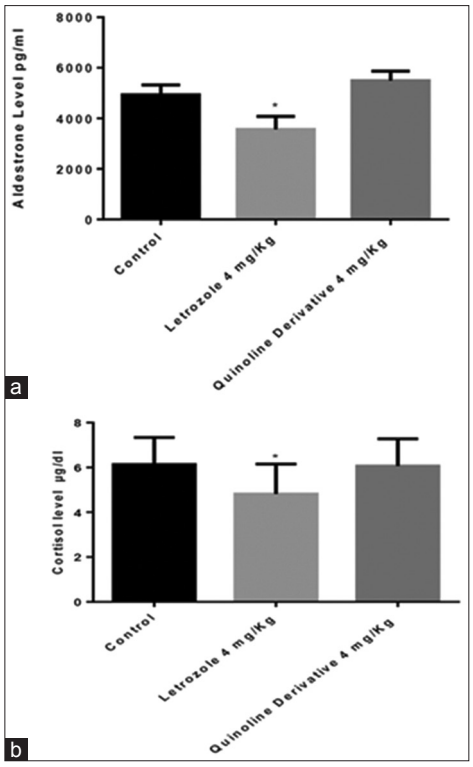

Figure 5: Effects of single oral doses of (a) quinoline derivatives and (b) letrozole on serum concentrations of cortisol and aldosterone as an *in vivo* aromatase selectivity assay. Male rats received a subcutaneous injection of 10 mg/kg of adrenocorticotropic hormone, and 16 h later, they were treated with 4 mg/kg of either compound 8b or letrozole (control group received normal saline). After 2 h, all animals were beheaded, and their whole blood was collected. Serum concentration of aldosterone and cortisol was measured by ELISA assay. The values are shown as mean ± standard error of the mean of determinations carried out of six rats in each group. ***$P \leq 0.001$

related to better interaction with amino acids present in the active site of aromatase. These quinolines 8a-g were more cytotoxic against MCF-7 cells in comparison with those of T47D. MCF-7 and T47D cell lines both are two estrogen-positive breast cancer cell lines and the amount of aromatase enzyme in MCF-7 cells is more than T47D cells, which may be due to their ability to inhibit aromatase or decrease aromatase activity. Furthermore, compound 8b that decreased the aromatase activity more than the others was not the most potent antiproliferative agent, suggesting other effects in addition to inhibition of aromatase activity.[15] Hence, the compound 8b (4-((1H-imidazol-1-yl) methyl)-2-(4-fluorophenyl)-8-phenylquinoline) among the other compounds (8a-g) was selected in this study for *in vivo* evaluation.

The *in vivo* effect of compound 8b has been studied by the measurement of the inhibition of androstenedione-induced uterine hypertrophy. In this method, androstenedione is aromatized to estrogen in the ovary that caused uterine hypertrophy. Therefore, inhibition of this uterine hypertrophy is considered as an index for aromatase inhibition *in vivo*. The results of this study showed that letrozole could significantly inhibit uterine hypertrophy that was the same as the previous reports that letrozole inhibits uterine hypertrophy with the ED_{50} (50% effective dose) 1–3 μg/kg.[16] Furthermore, these results indicated that the *in vivo* aromatase inhibitory potency of compound 8b was similar to letrozole that was in agreement with the results of *in vitro* studies. When the same doses of quinoline derivative 8b were administered, it did not show any significant effects on the serum concentration of either aldosterone or cortisol.

Previous studies showed that letrozole is highly selective for aromatase unlike first and second generation.[19] *In vivo* ACTH stimulation on the plasma concentrations of aldosterone and cortisol showed that compound 8b had no significant effect on either aldosterone or cortisol levels, even at a dose 400 times more than that required for *in vivo* inhibition of aromatase activity. Surprisingly, compound 8b showed more selective *in vivo* effects than letrozole in which letrozole (4 mg/kg) could significantly decrease the serum concentration of aldosterone and cortisol as compared to the control group.

CONCLUSION

In this study, we have described the *in vivo* evaluation of compound 8b as aromatase enzyme inhibitors. *In vivo* studies have shown that compound 8b is highly potent and selective AI which was similar to known AI letrozole and showed more selective *in vivo* effects than letrozole. Therefore, compound 8b can be considered as the new lead for further investigation to explore the more potent and more selective AIs with lower side effects compared to letrozole for hormone-dependent breast cancer.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Theriault RL, Carlson RW, Allred C, Anderson BO, Burstein HJ, Edge SB. Breast cancer, version 3.2013: Featured updates to the NCCN guidelines. J Natl Compr Canc Netw 2013;11:753-60.
2. Barry M, Kell MR. Enhancing the adjuvant treatment of hormone receptor positive breast cancer. Breast J 2009;15:194-8.
3. Su B, Díaz-Cruz ES, Landini S, Brueggemeier RW. Suppression of aromatase in human breast cells by a cyclooxygenase-2 inhibitor and its analog involves multiple mechanisms independent of cyclooxygenase-2 inhibition. Steroids 2008;73:104-11.

4. Altundag K, Ibrahim NK. Aromatase inhibitors in breast cancer: An overview. Oncologist 2006;11:553-62.
5. Miller WR. Background and development of aromatase inhibitors. In: Aromatase Inhibitors. Switzerland: Birkhäuser Verlag; 2006. p. 1-21.
6. Evans CT, Ledesma DB, Schulz TZ, Simpson ER, Mendelson CR. Isolation and characterization of a complementary DNA specific for human aromatase-system cytochrome P-450 mRNA. Proc Natl Acad Sci U S A 1986;83:6387-91.
7. Stanway SJ, Delavault P, Purohit A, Woo LW, Thurieau C, Potter BV, *et al*. Steroid sulfatase: A new target for the endocrine therapy of breast cancer. Oncologist 2007;12:370-4.
8. Wang M, Mickens J, Gao M, Miller KD, Sledge GW, Hutchins GD. Design and synthesis of carbon-11-labeled dual aromatase-steroid sulfatase inhibitors as new potential PET agents for imaging of aromatase and steroid sulfatase expression in breast cancer. Steroids 2009;74:896-905.
9. Kalidas M, Brown P. Aromatase inhibitors for the treatment and prevention of breast cancer. Clin Breast Cancer 2005;6:27-37.
10. Goss PE, Strasser K. Aromatase inhibitors in the treatment and prevention of breast cancer. J Clin Oncol 2001;19:881-94.
11. Chen S, Zhou D, Okubo T, Kao YC, Eng ET, Grube B. Prevention and treatment of breast cancer by suppressing aromatase activity and expression. Ann N Y Acad Sci 2002;963:229-38.
12. Bonfield K, Amato E, Bankemper T, Agard H, Steller J, Keeler JM, *et al*. Development of a new class of aromatase inhibitors: Design, synthesis and inhibitory activity of 3-phenylchroman-4-one (isoflavanone) derivatives. Bioorg Med Chem 2012;20:2603-13.
13. Pouget C, Yahiaoui S, Fagnere C, Habrioux G, Chulia AJ. Synthesis and biological evaluation of 4-imidazolylflavans as nonsteroidal aromatase inhibitors. Bioorg Chem 2004;32:494-503.
14. Pouget C, Fagnere C, Basly JP, Habrioux G, Chulia AJ. Design, synthesis and evaluation of 4-imidazolylflavans as new leads for aromatase inhibition. Bioorg Med Chem Lett 2002;12:2859-61.
15. Ghodsi R, Azizi E, Grazia Ferlin M, Pezzi V, Zarghi A. Design, synthesis and biological evaluation of 4-(Imidazolylmethyl)-2-aryl-quinoline derivatives as aromatase inhibitors and anti-breast cancer agents. Lett Drug Des Discov 2016;13:89-97.
16. Bhatnagar AS, Häusler A, Schieweck K, Lang M, Bowman R. Highly selective inhibition of estrogen biosynthesis by CGS 20267, a new non-steroidal aromatase inhibitor. J Steroid Biochem Mol Biol 1990;37:1021-7.
17. Blanco JG, Gil RR, Bocco JL, Meragelman TL, Genti-Raimondi S, Flury A. Aromatase inhibition by an 11,13-dihydroderivative of a sesquiterpene lactone. J Pharmacol Exp Ther 2001;297:1099-105.
18. Häusler A, Monnet G, Borer C, Bhatnagar AS. Evidence that corticosterone is not an obligatory intermediate in aldosterone biosynthesis in the rat adrenal. J Steroid Biochem 1989;34:567-70.
19. Haynes BP, Dowsett M, Miller WR, Dixon JM, Bhatnagar AS. The pharmacology of letrozole. J Steroid Biochem Mol Biol 2003;87:35-45.