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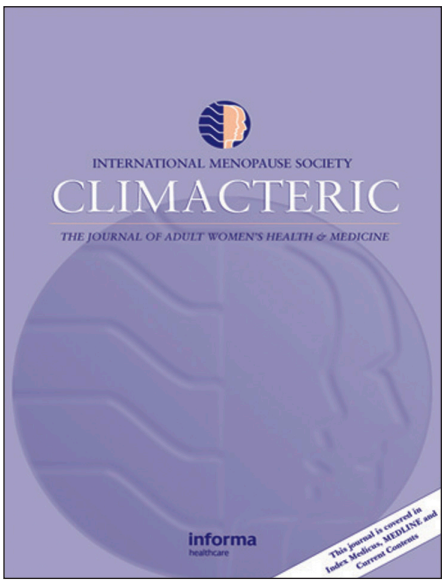

Efficacy and safety of a phyto-SERM as an alternative for hormone therapy

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ABSTRACT

In this review, we analyse the efficacy and safety of DT56a in the treatment of postmenopausal symptoms. Similar to all selective oestrogen receptor modulators (SERMs), DT56a demonstrates dual agonistic and antagonistic effects due to the synergy between its components. DT56a is referred to as a plant-origin SERM (phyto-SERM), and for this reason, its therapeutic capacity in postmenopausal women differs from other phytoestrogens used independently. Although interesting data on vasomotor symptom relief have been reported for DT56a, further clinical studies with a greater number of cases and a longer period of study are required to correctly identify its indications for use as an alternative hormone therapy, especially in preventing osteoporosis.

Efficacy and safety of a phyto-SERM as an alternative for hormone therapy

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Short title: review of phyto-SERM

ABSTRACT

In this review, we analyse the efficacy and safety of DT56a in the treatment of postmenopausal symptoms. Similar to all selective oestrogen receptor modulators (SERMs), DT56a demonstrates dual agonistic and antagonistic effects due to the synergy between its components. DT56a is referred to as a plant-origin SERM (phyto-SERM), and for this reason, its therapeutic capacity in postmenopausal women differs from other phytoestrogens used independently. Although interesting data on vasomotor symptom relief have been reported for DT56a, further clinical studies with a greater number of cases and a longer period of study are required to correctly identify its indications for use as an alternative hormone therapy, especially in preventing osteoporosis.

Key words: phytotherapy, phytoestrogens, menopause, lignans, SERM

INTRODUCTION

Postmenopausal oestrogen deficiency produces vasomotor symptoms, vaginal dryness and an increased risk of osteoporosis. Oestrogen- and gestagen-based hormone therapy (HT) is the first option to alleviate these ailments. However, the potential for side effects negatively impacts the use of HT and instead promotes the use of alternative treatments.

Oestrogen-like natural substances (phytoestrogens) or oestrogen-rich diets can provide soft hormonal effects. There are three main types of phytoestrogens in dietary and pharmacological forms, including isoflavones, coumestans and lignans, and there is a growing interest to identify whether better results are obtained when two or more of such compounds are consumed together compared to individually.[1]

DT56a (Femarelle) is a natural medication that contains a variety of phytoestrogens originating from tofu. Treatment with DT56a affects the bone, vascular tissue and nervous system in a similar manner as oestrogen but delivers an antagonist effect in breast and uterus tissue. These dual effects are why DT56a is classified as a selective oestrogen receptor modulator (SERM), specifically a phyto-SERM.[2] The objective of this review was to analyse the efficacy and safety of DT56a in the treatment of menopausal problems as an alternative to HT.

METHODS

Selection of studies

We searched the PubMed database for all articles, in all languages, published in peer-review journals through December 2013 using the search strategy displayed in Appendix A. First, we included articles that there were relevant to show the effects of DT56a (Femarelle). Articles in which combined phytoestrogens treatment or prevention of menopausal symptoms or consequences were also included. Inclusion criteria were: any experimental or clinic study focusing on DT56a.

Assessment of study quality and data synthesis

We followed PRISMA guidelines for systematic reviews (http://www.prisma-statement.org/statement.htm). PICOS (population, interventions, comparators, outcomes, studies design) criteria were formulated a priori to guide the review's scope and the search procedure, selection and synthesis of the literature. The authors independently conducted the search and screened studies for inclusion, extracted and checked the data, and synthesized the findings. The authors independently determined the adequacy of the design of the studies and their main methodological characteristics in order to ascertain the validity of eligible research. Disagreements were resolved by discussion and consensus. When duplicates of a study were found, we selected which was more detailed or pieced together data from the multiple reports indicating the corresponding references.

RESULTS

The literature search revealed 17 studies, but the current systematic review was based finally on 13 publications. Full articles that met the inclusion criteria were reviewed in detail. Data items to be considered were discussed by the review authors and appear in Table 1. Other relevant papers were used for reference list purposes.

Mechanism of action

DT56a is a tofu derivative containing at least 11 types of phytoestrogens, such as isoflavones, lignans and coumestans. Laboratory studies have demonstrated the SERM action of DT56a, showing increased levels of the creatinekinase (CK) enzyme in the cerebrovascular and bone tissues but not in

the uterus.[3,4] This marker has been used to assess activation of the oestrogen receptor (ER) and, like other phytoestrogens, has also been used to confirm the affinity of DT56a to ER-β (100,000-fold higher than that for ER-α, thus demonstrating its limited oestrogenic action in the endometrium and breast.[5] Additionally, DT56a demonstrates an enzymatic inhibition capacity of hormone metabolism and shows a significant antioxidant effect.[6]

Efficacy

1. Efficacy against vasomotor symptomatology

Two observational studies have analysed the efficacy of DT56a in terms of an improvement in menopausal symptoms. In the American study, which included 80 postmenopausal women over a 12-month period and compared two different DT56a doses (644 and 344 mg/day), vasomotor symptoms were decreased in up to 75% of subjects, headaches in 65% and joint pain in 70%, with no breast, endometrial or hormonal changes following its use.[7]

In the Greek study, two groups of 89 symptomatic postmenopausal women received DT56a or HT randomly, and the results were compared between the treatment group and a control group of women who rejected the treatment. The results revealed a decrease in vasomotor symptoms with both treatment respect to control; but it seems that HT was better than DT56a (mean difference in Kupperman score, DT56a group: -3.98, HT group -5.601, no treatment group +1.76, p-value <0.001).[8]

An Italian study in animals showed that DT56a had an effect on the opioid system similar to oestrogens, which may explain the clinical effects of this phyto-SERM on menopausal symptoms.[9]

2. Actions on the bone

Animal research studies with DT56a have revealed similar effects in bone and cartilage when comparing DT56a to other forms of oestrogen. An increase in CK was responsible for these oestrogenic effects, as measured in the cancellous and cortical bone.[10]

In another study from the same group that used osteoblast cultures from postmenopausal women, DT56a was active in normoglycaemic and hyperglycaemic environments. Of note, this effect differed from that observed with oestradiol, which loses its efficacy in hyperglycaemic environments. This property of DT56a may be useful for improving the bone health of postmenopausal women with alterations in carbohydrate metabolism.[11]

Only one article has evaluated the clinical effect of DT56a on the bone mineral density (BMD) of postmenopausal women. This one-year prospective study used two different doses of DT56a (644 and 344 mg/day) and showed that, compared to the baseline measurement, the vertebral bone BMD increased only with the 644 mg/day dosage.[2]

3. Actions on the cardiovascular system

The effect of DT56a on vascular tissue has been demonstrated in animal research studies. In ovariectomised rats, increased levels of CK produce an oestrogenic effect similar to that observed in the bone and vascular tissue of the nervous system.[12]

In an observational study of 32 postmenopausal women with severe vasomotor symptoms, including 7 women with some type of thrombophilia, DT56a did not lead to changes in platelet function, which indicates that this compound may represent a useful alternative to HT in patients with thrombotic risk.[13]

Safety

The special affinity of DT56a to ER-β leads to minimal effects in tissues where ER-α predominates (uterus, ovary and breast). In fact, DT56a increases CK (oestrogenic effect) levels in the bone and vascular tissue, but not in the uterus, as observed in animal research studies. One of these animal trials showed that bone and uterine CK levels were increased with oestradiol and a high dose of injected DT56a, while the bone CK (but not uterine CK) level was increased when DT56a was administered orally in low doses. In the same study, when prescribing raloxifene, the action of oestradiol and DT56a was blocked at all levels.[14]

In another laboratory study using ovariectomised rats, Tofupill supplementation led to much lower oestrogenic effect compared to the effects of conjugated equine oestrogens on the reproductive tract (vaginal epithelium, myometrium and uterine weight).[15]

Regarding mammary safety, in studies using MCF-7 breast cancer cell lines, cellular proliferation was not observed with the typical dose of DT56a compared to the use of oestrogens.[16]

Furthermore, other experimental animal studies suggest that DT56a may provide liver protection through a mechanism that involves several components of the cellular immune response.[17]

Two previous studies from Yoles *et al* [2,7] provided data on endometrial safety using two doses of DT56a. In these studies designed to analyse the effects of two doses of DT56a on BMD and the symptoms of healthy postmenopausal women, changes in endometrial thickness or hormone levels during the year-long duration of the study were not observed at either dose.[2,7]

Drug interactions

In the current literature, DT56a has not been associated with any serious adverse effects, and no data are available regarding its interaction with other drugs.

DISCUSSION

To the best of our knowledge, this is the first review on the use of DT56a (Femarelle). With the objective of considering DT56a as an alternative to HT, this review analysed experimental and clinical data for this phyto-SERM, presenting data on its efficacy for the improved relief of vasomotor symptoms, its effect on halting postmenopausal bone loss and its safety profile for hormone-dependent organs. In general, our findings agree with the current "trend" suggesting that the use of a combination of different phytoestrogens is more effective and safe than using a single form.[1]

It is widely accepted that a considerable percentage of postmenopausal women experience significant changes in their quality of life due to hypoestrogenism.[18] Although the combination of natural products to relieve menopausal symptoms has been increasingly studied, the precise mechanism that mediates the effects of hypoestrogenism remains unknown. However, a multifactorial hypothesis is generally accepted, especially when trying to explain which neurotransmitters (dopamine, noradrenaline or endogenous opioid peptide) are implicated in changes to the hypothalamic thermoregulatory centre.[19]

It is generally agreed that HT is the first option for menopausal symptom relief, although the side effects, or the fear of suffering from them, has decreased the use of HT, and alternative measures have been developed related to the pharmacological and dietetic intake of phytoestrogens.[20] The use of such alternative treatment options varies depending on the analysed region of the world, in the same way that there are differences in digestive processes and intestinal flora between populations. For example, Asian diets are high in isoflavones from soybeans, while Western diets include vegetables with high levels of lignans.[21] In addition, the consumption of these compounds is low in Europe, especially in southern countries.[22]

Although the exact composition of DT56a remains unknown, this compound is derived from tofu and contains more than 11 types of phytoestrogens. Moreover, the joint actions of such components may be related to the plant origin of this SERM, or its classification as a phyto-SERM.[2] However, the lack of understanding of this substance makes its management confusing and leads to studies that analyse the isolated or combined effect of its components. The majority of previous research and the medical indications for phytoestrogens during menopause have been obtained in isoflavones, although data on the efficacy of lignans and coumestans is also available.[23] In addition, these compounds all present common structural characteristics, such as 17β-oestradiol, which is recognised by the ER (mainly ER-β).[24]

The differences between these compounds likely depend on structural issues and bioavailability, which cases some compounds to behave individually as SERMs.[25,26] In this regard, enterodiol and enterolactone, which are lignans metabolites, demonstrate an oestrogenic effect on bone [21, 27] but an anti-oestrogenic effect on breast cancer cell cultures.[28] Other compounds (acteoside and martynoside) have shown a significant anti-proliferative effect on the endometrium.[29] Additionally, in general, lignans demonstrate the same antioxidant activity as isoflavones[30], whereas for coumestans, these compounds show greater affinity to the ER than genistein[31] and also impact enzymes involved in hormone metabolism.[32]

Previous studies have also reported the combined effect of isoflavones and lignans. In particular, a study performed in 80 recently menopausal women observed a significant reduction in menopausal hot flashes after 3 months of combined therapy with isoflavones, lignans and black cohosh compared to a control group that received only calcium tablets.[33] Although this was not a direct comparative study, it can be inferred that this synergism resulted in more significant and more rapid relief of hot flashes.

Meanwhile, articles directly analysing the efficacy of DT56a on vasomotor symptoms have reported more rapid and continuous relief (2 to 4 weeks) compared to that observed in studies analysing only the effect of isoflavones. This difference is likely due to the phytoestrogen combination of different pharmacokinetics. This synergism has also been observed in the treatment for vaginal atrophy, although this application may require further investigation.[34]

Regarding osteoporosis, which affects one-third of all postmenopausal women, all treatments capable of ameliorating this condition should be considered.[35] Although there is a lack of sufficient evidence to affirm that phytoestrogens may prevent the occurrence of osteoporotic fractures, some bone protection has been observed.[36] Moreover, it has been suggested that some compounds (such as alpha-linolenic acid) show added bone protection arising from the synergism between their oestrogenic effect and antioxidant action.[37]

The use of DT56a has also shown to improve bone mass in healthy postmenopausal women with vasomotor symptoms.[7] Although a randomised controlled trial (RCT) is required to study women with a low BMD, the results of this prospective study are sufficiently promising to suggest DT56a as an alternative to HT in the prevention of osteoporosis. Additionally, this article warns that a dose greater than 644 mg/day or duration longer than 6 months is required for the effective maintenance of BMD.[2]

Research studies on the effect of DT56a on bone show that the activity of this phyto-SERM is maintained in normoglycaemic and hyperglycaemic environments. This result differs from that seen with oestradiol, which loses its effectiveness in hyperglycaemic environments, and this result could be applied to improve the bone health of postmenopausal women with an alteration in carbohydrate metabolism.[11]

Many benefits and risks of menopause treatments are related to cardiovascular effects. Vascular health is also affected by postmenopausal hypoestrogenism, although it seems that the risk/benefit balance of HT is highly dependent of the previous arterial health state. Therefore, HT may be valuable in women without vascular pathology during the "window period" in the immediately years following menopause, rather than in the presence of atheromatous lesions or in women older than 65 years of age.[20] Similarly, cardiovascular protection can also be conferred from treatment with phytoestrogens due to their binding capacity to the ER without the reported side effects, mainly thrombogenic, described for HT.[38] Furthermore, the findings of an observational study on 32 postmenopausal women with severe vasomotor symptoms, including 7 women with some degree of thrombophilia, who received DT56a and the absence of any alterations in platelet function were interesting. However, additional studies on this subject are required, as this may represent an advantage of DT56a and a clear alternative to HT in patients with a thrombotic risk.[34]

Aside from the thrombotic risk, the safety concern for phyto-SERMs has focused on their effects on the uterus and breast. Laboratory studies have shown that DT56a does not have an oestrogenic effect in the uterus and does not have an effect on cellular proliferation in cultures of mammary cancer cells. Moreover, if we apply the findings of studies analysing the mammary and endometrial actions of phytoestrogens, it is possible that these compounds could have antitumor effects. Therefore, lignans, due to their anti-oestrogenic uterine effects, may reduce the risk of endometrial adenocarcinoma (IRR 0.93, 95%CI 0.84, 1.04).[39] However, only one clinical study has measured the safety of two doses of DT56a[7], in an attempt to analyse the effects of varying doses of DT56a on the BMD of healthy postmenopausal women. This year-long study did not observe changes in endometrial thickness or hormone levels following treatment with either dose.[2]

Safety data on phytoestrogen intake regarding the mammary glands have also been reported, even in breast cancer survivors.[40] Other studies have also reported a reduction in the risk of cancer, although these data are limited and controversial.[41] Moreover, it remains unclear whether the use of DT56a,

together with tamoxifen or anastrozole, would produce interactions and reduce the benefits of treatment.[42] Nevertheless, according to a breast cancer survivors' treatment guide, it is recommended to avoid the intake of phytoestrogens during breast cancer treatment.[43]

Finally, severe adverse effects of DT56a were not reported in the reviewed articles, and data on possible interactions with other drugs are not available. Again, this information can only be inferred from that previously published for other phytoestrogens. Thus, most side effects described include mild gastrointestinal alterations. Following a high treatment dose and when administered with nonsteroidal anti-inflammatory drugs, vaginal bleeding has been observed. In addition, because bacterial flora is necessary to transform these compounds into their active forms, joint intake with antibiotics may decrease their effect.[44] It should also be noted that isoflavones are enzymatic inhibitors of the cytochrome P_{450}, which in theory would maximise the toxicity of some substances when taken jointly. For example, there is an in vitro interaction between alcohol and daidzin that inhibits the aldehyde dehydrogenase enzyme involved in the metabolism of alcohol, and interactions between ipriflavone (a synthetic derivative of the isoflavones) and theophylline, phenacetin or tolbutamide that may reduce the metabolism and elimination of these substances. Similarly, phytoestrogens also behave as enzymatic inductors, which could facilitate the clearance of drugs such as thyroid hormones, thereby decreasing their therapeutic effect.[45]

In previous performed observational studies, side effects of treatment with phytoestrogens and other drugs were not observed in postmenopausal women.[46]

The main limitations of this review are related to the composition of DT56a, which was not sufficiently detailed, and its restricted use during the postmenopausal period. Additionally, the scarcity and heterogeneity of previous studies impede the potential usefulness of DT56a for relieving other common symptoms related to menopause, such as arthralgia, insomnia and vaginal atrophy. One of the main attractions of DT56a, i.e., the possibility of osteoporosis prevention, also presents limitations because this capacity could not be established in follow-up studies lasting less than 2 years. However, these limitations also open the door to future investigations, mainly in the areas of design, efficacy and safety. In particular, future studies should be mainly designed as RCTs, and efficacy analysis should focus on other conditions of postmenopausal woman, mainly vaginal atrophy, other psychological symptoms and the prevention of osteoporosis. Moreover, there is a need to extend the duration of such studies to longer than 2 years. Finally, it would be interesting to evaluate the dose-response relationship between DT56a (and all component phytoestrogens) and the risk of developing breast cancer.

CONCLUSION

In conclusion, DT56a demonstrates agonistic and antagonistic effects depending on the target tissue, which is why this compound is considered a phyto-SERM. Its therapeutic efficacy in postmenopausal women differs from that of other phytoestrogens used independently, likely due to the synergic actions of its components. Although interesting data on the relief of vasomotor

symptoms are available, additional studies with greater numbers of cases and longer durations are required to correctly set the indications for DT56a as an alternative to HT, especially in the prevention of osteoporosis.

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APPENDIX A

Search strategy

("DT56a"[Supplementary Concept] OR "DT56a"[All Fields] OR "dt56a"[All Fields]) OR ("DT56a"[Supplementary Concept] OR "DT56a"[All Fields] OR "femarelle"[All Fields]) OR phytoserm[All Fields]

Table Legends

Table 1. summary of laboratory studies

	OBJECTIVE	METHODS	MAIN RESULTS
Somjen et al., 2003[12]	estrogenic effects of DT56a	Effects of DT56a and DT56 + Raloxifene on vascular animals tissues (aorta and the left ventricle of the heart).	DT56a has similar effects on vascular tissues like that of E2, probably mediated via common receptor(s).
Somjen et al., 2003[14]	estrogenic effects of DT56a	Effects of E2, DT56a and DT56+ Raloxifene on bone and cartilage of immature or ovariectomized female rats, by measuring the changes in the specific activity of the BB isozyme of CK.	DT56a acts as a SERM stimulating skeletal tissues without affecting the uterus.
Somjen et al., 2005[10]	estrogenic effects of DT56a	Effects of long term treatment (two months) with DT56a on the skeletal tissues of intact and ovariectomised adult rats.	DT56a was as effective as E2 in reversing the bone changes caused by Wistar ovariectomized (OVX) in rats.
Somjen et al., 2006[6]	estrogenic effects of DT56a	Effects of DT56a in vitro on human-derived bone cultured Ob, by measuring its effects, at different concentrations, on DNA synthesis, CK and alkaline phosphatase (ALP) specific activities as well as changes in intracellular [Ca(2+)] concentrations.	DT56a stimulated sex-specifically female-derived Ob, indicating its unique nature compared to the compounds currently used for postmenopausal osteoporosis by being bone-forming and not only an anti-resorptive agent.
Somjen et al., 2011[11]	estrogenic effects of DT56a	Effects of DT56a in vitro on human-derived bone cultured Ob when grown in high glucose concentration (HG).	HG increased constitutive CK but, the response of CK activity and DNA synthesis to E2 treatment was reduced. In contrary, DT56a was found to be active (as measured by CK activity and DNA synthesis) in both normal and high. HG decreases the hormonal responsiveness and might block important effects of estrogenic compounds, most likely contributing to their decreased skeletal preserving properties in hyperglycemic women. In Ob from post-menopausal women grown in HG, ERs mRNA expressions were unchanged. On the other hand, in Obs from pre-menopausal women HG increased ERs mRNA expressions.
Somjen et al., 2007[4]	Interaction between DT56a and E2	Interaction between DT56a and E2, at supra physiological doses, in different tissues in both intact and ovariectomized female rats, as well as in human cultured vascular and bone cells.	DT56a is a SERM: it stimulated different parameters similar to E2, but when given simultaneously, at supra physiological doses, inhibited these E2's effects.
Oropeza et al., 2005[15]	estrogenic effects of DT56a	Food supplement (3.4 or 10.2 mg/kg) and conjugated equine estrogens (CEE, 31 or 100 mcg/kg) were orally administered, daily during 14 days to ovariectomized rats. At the end of treatment, the following determinations were done: dry and wet uterine weight, vaginal epithelium condition, and uterine serotonin-induced contractile response. A group treated with 17beta-estradiol was included as control for serotonin-induced contractile response.	Food supplement did not display clear estrogenic effects on vaginal epithelium, uterine weight or myometrial sensitivity to serotonin, whereas high doses of conjugated equine estrogens showed estrogenic action.
Shabat et al., 2013[17]	metabolic and immunological effects of DT56a	DT56a was orally administered to mice in three animal models: leptin deficiency, high-fat diet supplementation and immune-mediated hepatitis. Liver damage and immunological status were assessed.	Oral administration of DT56a promotes a hepatoprotective effect associated with an alteration in the distribution of Tregs and NKT cells.
Pluchino et al., 2009[9]	neuroendocrine effect of DT56a	Five groups of Wistar ovariectomized (OVX) rats received one of the following treatments: oral DT56a administration at doses of 6, 12, 60, and 120 mg kg(-1) day(-1) or estradiol valerate at a dose of 0.05 mg kg(-1) day(-1) for 14 days. One group of fertile and one group of OVX rats receiving placebo were used as controls. The	This study demonstrated that DT56a positively affects brain neurosteroidogenesis and the opiatergic system: DT56a exerts an estrogen-like effect on selective areas related to mood, cognition, and homeostasis control, presenting a specific pattern of interaction with the brain function.

		concentration of allopregnanolone was assessed in the frontal and parietal cortex, hippocampus, hypothalamus, anterior pituitary, and serum, whereas the content of beta-endorphin was evaluated in the frontal and parietal cortex, hippocampus, hypothalamus, neurointermediate lobe, anterior pituitary, and plasma.	

CK: creatine kinase; E2: estradiol-17beta; ER: estrogen receptor; Ob: osteoblasts, SERM: selective estrogen receptor modulator

Table 2. Summary of clinical studies

	OBJECTIVE	SUBJECTS AND METHODS	RESULTS	CONCLUSIONS
Labos et al., 2013[8]	Menopausal symptoms	Prospective study with 89 post-menopausal women with climacteric symptoms, randomly assigned to receive either DT56a (n=27) or oral low dose continuous combined HT (n= 26). Symptomatic women not wishing to receive any treatment served as controls (n= 36). Kupperman index, serum lipids and lipoproteins, calcium, as well as bone mineral density (BMD), endometrial thickness, and mammography were assessed at baseline and at 12 months.	Patients receiving HT and DT56a showed a significant and independent decrease in menopausal symptoms (mean difference in Kupperman score, DT56a group: -3.98, HT group -5.601, no treatment group +1.76, p-value <0.001). Lumbar spine BMD T-score was significantly lower in women receiving no treatment, as opposed to the two treatment arms which showed no significant change (No treatment, baseline: -0.60, final: -0.85, p=0.001; HT, baseline: -84, final -0.99, p=0.79; DT56a, baseline -0.51, final: -0.76, p=0.75). No differences in femoral bone density, ET or mammography classification were detected in any of the treatment arms. Likewise, serum lipids or lipoproteins did not differ between the three groups.	DT56a decreased menopausal symptoms significantly and in the same degree as HT.
Nachtigall et al., 2011[13]	effect of DT56a on platelet function in normal and thrombophilic women	The Platelet Function Analyzer-100 was used to asses platelet reactivity at baseline and after 8 weeks of treatment with DT56a (644 mg/d) in 25 symptomatic postmenopausal women with normal clotting times and 7 symptomatic women with shortened clotting times (<61 s).	All participants reported improved symptoms during the treatment period. No significant change in closure times was found in the normally clotting participants after 3 or 8 weeks of DT56a therapy (P > 0.26). No significant change in closure time was seen in the seven thrombophilic women after 3 or 8 weeks or 1 year of treatment (P >0.26). The regression curve for measures over time was not significant (P =0.26).	DT56a did not adversely affect platelet reactivity as measured by PFA closure times in symptomatic thrombophilic postmenopausal women or normal controls.
Yoles et al., 2003[2]	efficacy of DT56a in preserving BMD in postmenopausal women.	98 healthy, postmenopausal women randomly allocated, on a double-blind basis, to receive either 644 mg/d DT56a (study group) or 344 mg/d DT56a supplemented with calcium (low-dose group) for 12 months.	BMD had increased in the study group by 3.6% in the lumbar spine (P = 0.039) and by 2.0% in the femoral neck (NS). In the low-dose group, BMD had decreased in the lumbar spine by 0.6% (NS) and by 0.6% in the femoral neck (NS). Comparison of the change in bone density between the groups yielded a significant difference for the lumbar spine (P = 0.037). Neither group showed a change in endometrial thickness and sex hormone levels nor reported any side effects of treatment.	DT56a increases BMD without unwanted estrogenic effect.
Yoles et al., 2004[7]	efficacy and safety of the two doses of DT56a	80 postmenopausal women were randomly allocated to receive either the standard dose (SD) or low dose (LD) of Femarelle (644 mg/day vs 344 mg/day). A detailed Kupperman index for each patient was completed. 12 months.	In both groups there was a significant reduction in the Kupperman index following 12 weeks of treatment, which was sustained throughout the 12 months (p < 0.01). 76% of the patients in the SD group reported a decrease in vasomotor symptoms and 78 eight % in the LD group (NS). This decrease was sustained following 12 months of treatment. There was no change in TSH and sex hormone levels or endometrial thickness during the study period.	Menopausal symptoms were reduced similarly by LD and SD, however for the combined treatment of menopausal symptoms and osteoporosis the standard dosage of 644 mg/day of DT56a is needed.

BMD: bone mineral density; HT: hormonal treatment;