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Full Length Research Paper

Oestrogenic properties of the ethanolic extract of *Fernandoa adolfi friderici* (Bignoniaceae) stem bark

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Fernandoa adolfi friderici **is used traditionally to treat menopause related symptoms. The effects of the ethanolic extract of this plant in ovariectomized (OVX) rats were investigated. 40 of 50 rats were OVX and separated into four groups of 10 each. 84 days after ovariectomy, animal groups were started on one of four treatments: ethanol/0.09% NaCl (control group), oestrogen, extracts of *F. adolfi friderici* (FA 150 and 300 mg/kg), respectively. 5 animals from each group were terminated after 14 and 28 days of treatment, respectively and vaginal smears, vaginal/uterine wall cytology, lipid profiles and bone mass and histology were studied. *F. adolfi friderici* treated OVX rats had superficial cells which were absent in vaginal smears of control animals. The former had improved vaginal epithelial thickness after 28 days of treatment (55.8±8.6 versus 13.2±0.8) compared to control animals. *F. adolfi friderici* extracts also improved serum triglyceride (27.2%/28.2% reduction versus 73% increase in controls) and high density lipoprotein (HDL) (0.78±0.01/0.98±0.09 g/L versus 0.6±0.05 g/L) levels while improving bone architecture and preventing bone resorption. *F. adolfi friderici* may be the source of a useful phytoestrogen for the complementary management of menopausal symptoms.**

Key words: *Fernandoa adolfi friderici*, ovariectomized, phytoestrogen.

INTRODUCTION

Many women experience one or more unpleasant symptoms of menopause, the most common being hot flashes, depression, mood swings, sleeping disorders and vaginal dryness. These symptoms have been largely attributed to low levels or absence of oestrogens (Stevenson, 2011). Hormone replacement therapy (HRT) has been useful in relieving most of these symptoms as well as reducing the risk of osteoporosis (Al-Anazi et al., 2011). However, HRT using synthetic oestrogens significantly increases the risk of developing cardiovascular diseases as well as breast cancer (Chlebowski and Anderson, 2012). Women are therefore, increasingly reluctant to use HRT preferring herbal alternatives for menopausal symptoms (Pitkin, 2012). Several herbs and foods have been shown to contain phytoestrogens which are reported to exhibit both oestrogenic and anti-oestrogenic effects (Yoo et al., 2005; Tice et al., 2003). The two most studied phytoestrogens genistein and daidzein (Ososki and Kennelly, 2003; Boue et al., 2003) were initially isolated from the soy plant and formulated for prescription. Although phytoestrogens display oestrogenic effects, they are not as potent as synthetic oestrogens in their oestrogenic actions and are therefore considered to be safer alternatives (Chearskul et al.,

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2004). Consequently, researchers are in the search for phytoestrogens which would be useful in preventing several symptoms of menopause, improving the quality of life of menopausal women without the risk associated with HRT. *Fernandoa adolfi friderici* is a medicinal plant used in rural communities in Cameroon to control excess bleeding following childbirth as well as menopause related health problems. In this study, we investigated the medicinal properties of ethanolic extracts of the stem bark of *F. adolfi friderici* (FA) on some symptoms of ovariectomy in rats.

MATERIALS AND METHODS

Plant

F. adolfi friderici is a Bignoniaceae which can grow up to a 20 m high tree. It is generally found in the so called *terra firma* of the forest between Congo, Cameroon and Gabon (Harris and Wortley, 1984). The plant samples were collected in Lomie (Eastern Province of Cameroon) in February 2006. Samples were identified and authenticated at the Cameroon National Herbarium in Yaoundé where a voucher specimen (no. 4833/SRFK) was deposited. The air-dried and ground stem bark (1000 g) was macerated in 95% ethanol at room temperature for 72 h. The solvent was recovered using a rotary evaporator under reduced pressure. The mass of crude extract obtained was 44 g. Extract of *F. adolfi friderici* was dissolved in ethanol/0.09% NaCl (1:9) for administration to animals.

Drugs

17ß-estradiol was obtained from Progynova®, France and was referred to as oestrogen.

Animals

Juvenile female Wistar rats, 2 to 3 months old were obtained from the regular breeding facility of the Laboratory of Animal Physiology, University of Yaoundé I (Cameroon). Animals had free access to soy-free rat chow and water.

Experimental

Sexually mature female rats (50) were used. 10 rats served as the baseline control and were not OVX nor given any treatment. The remaining 40 rats were bilaterally OVX under ketamine and diazepam anaesthesia. 84 days after ovariectomy, rats were randomly assigned to one of the 4 groups treated orally with the vehicle, oestrogen (1 mg/kg), *F. adolfi friderici* (150 mg/kg) or *F. adolfi friderici* (300 mg/kg) daily for 28 days. Animals were weighed once every week. On day 14 of treatment, 5 rats were randomly selected from each group and terminated with an excess dose of ether. On day 28, the remaining animals were terminated and samples were collected from animals (blood, vagina, uterus and femur) for other tests.

Vaginal smears

Vaginal cytology was determined for all rats at the start of all experiments in order to establish the reproductive maturity of rats used in this study. Smears were taken from rats by flushing the vagina with of 0.5 ml of 0.09% NaCl. In the first series of experiments, an unknown quantity of smear was deposited onto a glass slide, fixed with methanol and stained with Giemsa-Maygründwald. The relative abundance of each cell type was determined by manual counting using light microscopy. Vaginal cytology was again performed on days 84 (12 weeks after ovariectomy), 98 (end of first treatment phase) and 112 (end of the second treatment phase). Ethanol was used to fix 100 µl of smears which were then stained using the Papanicolaou method. Parabasal, intermediate and superficial cells were counted using a light microscope.

Serum lipoproteins

Blood samples were collected at the beginning of the experiment to establish baseline data, then on days 84, 98 and 112 after ovariectomy (OVX). Blood was centrifuged at 3400 rpm at 5°C for 10 min. Serum total cholesterol, high density lipoprotein (HDL)-cholesterol and triglycerides levels were determined enzymatically using the fully automated Cobas Mira S analyzer with reagent/kits purchased from Biolabo (France), as per manufacturers' instructions. Levels of low density lipoprotein (LDL)-cholesterol were calculated from the concentration of total cholesterol, HDL-cholesterol and triglycerides.

Fresh bone weight

The femur of the right hind limb was delicately separated from the surrounding tissue and weighed using an analytical balance (Mettler ML303). The bones were subsequently fixed in 10% formaldehyde for histological analysis.

Histological analysis of endometrial, vaginal issues

Endometrial and vaginal epithelial thickness as well as bone tissue structure were assessed from 5 µm sections of paraffin embedded samples of these tissues followed by hematoxylin-eosin staining. Microscopic analysis of slides was made with the aid of an AxiosKop 40 microscope linked to a computer to which images were transferred, processed and analysed with MRGrab 1.0 and AxioVision 3.1 software from ZEISS Hallbermoos, Germany.

Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 10.0 was used for statistical analyses. One-way analysis of variance (ANOVA) and post-hoc multiple comparisons were performed by the non parametric Mann Withney U-test to reveal any significant differences between treatment groups and control. Results were expressed as means ± standard error. For all tests, statistical significance was defined as $p<0.05$.

RESULTS

Vaginal cytology

Vaginal smears on day 84 after ovariectomy showed a prevalence of parabasal cells in OVX rats compared to age matched non-OVX rats indicating atrophy of the vaginal epithelium. Oestradiol and *F. adolfi friderici* increased the number of superficial cells in vaginal smears after 14 and 28 days of treatment, respectively. However, the superficial cell scores were significantly

Table 1. Changes in cell types in vaginal smears of treated and untreated ovariectomized (OVX) rats.

Experimental day	Treatment group	Cell types/µl		
		Parabasal cells	Intermediate cells	Superficial cells
Day 98	Control	91±27	n.d	n.d
	Oestrogen	99.4±38	104±48	745±23
	FA 150 mg/kg	n.d	n.d	24±4
	FA 300 mg/kg	n.d	n.d	25±9
Day 112	Control	844±153	n.d	n.d
	Oestrogen	995±249	385±74	407±99
	FA 150 mg/kg	n.d	n.d	44±15
	FA 300 mg/kg	n.d	n.d	47±13

n.d = not detectable.

higher in the oestrogen treated rats. Importantly, only vaginal smears from oestrogen treated OVX rats had intermediate cells (Table 1).

Uterine wet weight

A significant decrease in uterine wet weight was observed 98 days after ovariectomy from 2255 ± 58 to 297 ± 20 mg/kg (87% decrease) and from 2377 ± 143 to 319 ± 30 mg/kg (86.6%) 112 days after ovariectomy. Oestrogen significantly ($p<0.01$) increased (372 and 377%) uterine wet weight after 14 and 28 days of treatment, respectively. At a dose of 150 mg/kg/day, *F. adolfi friderici* induced an initial decrease in uterine wet weight which improved after 28 days of treatment, though the difference was not significant (Figure 1). On the other hand, 300 mg/kg of *F. adolfi friderici* failed to improve uterine wet weight in OVX rats.

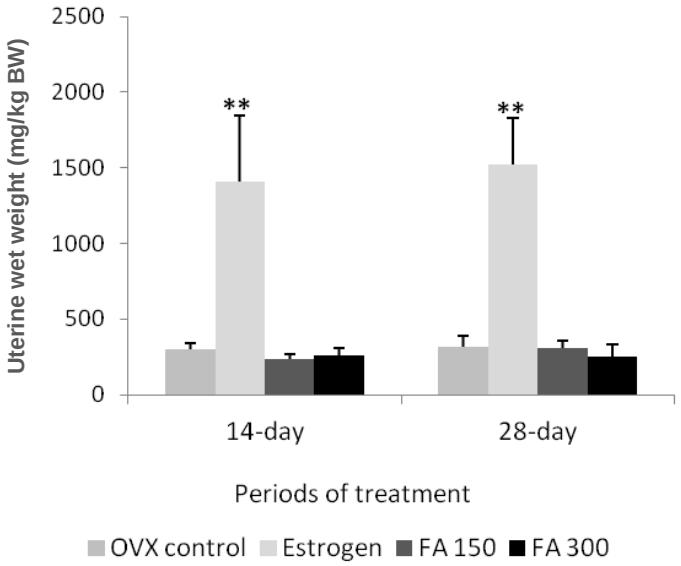

Figure 1. Effect of treatments on uterine wet weight of ovariectomized rats. OVX control = non treated ovariectomized rats; Oestrogen = oestrogen treated rats; FA 150 = 150 mg.kg of FA extract treated rats; FA 300 = 300 mg/kg FA extract treated rats. BW = body weight; **$p<0.01$.

Effects of FA treatment on vaginal and uterine epithelial thickness

Rat vaginal epithelial thinning increased significantly in all treatment groups between days 98 and 112 after ovariectomy. However, after 14 days treatment, the oestrogen group showed significant ($p<0.01$) increase in vaginal epithelial thickness (55.8±8.6 versus 13.2±0.8) while both doses of *F. adolfi friderici* failed to prevent vaginal wall thinning. However, after 28 days of treatment, both oestrogen and 300 mg/kg *F. adolfi friderici* significantly ($p<0.01$ and $p<0.05$) improved vaginal epithelial thickness (32.8±6.7 and 13.1±1.6, respectively) compared to the untreated group (Figure 2a). On the other hand, uterine epithelial thickness was significantly ($p<0.01$) increased by oestrogen treatment on both days 14 and 28 of treatment. Extracts of *F. adolfi friderici* failed to increase vaginal thickness; in fact, *F. adolfi friderici* 300 mg/kg rather induced a non-significant decrease in uterine epithelial thickness compared to OVX control animals (Figure 2b).

Effect of treatments on blood lipid profiles

Serum triglycerides

Serum triglyceride levels increased significantly (75.3%; $p<0.01$) in all OVX rats. Fourteen days of treatment with *F. adolfi friderici* significantly ($p<0.01$) reduced serum triglyceride levels (*F. adolfi friderici* 150 mg/kg: 27.2%; *F. adolfi friderici* 300 mg/kg: 28.2%.) while the effects of oestrogen were only marginal. Prolonged treatment (28

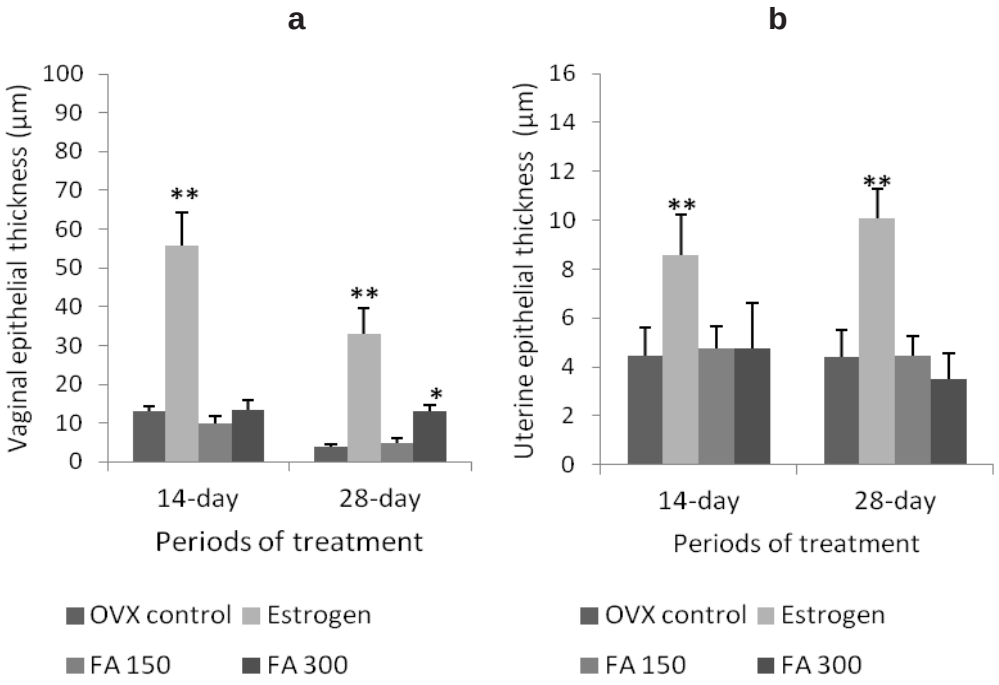

Figure 2. Effect of long term treatments on vaginal (a) and uterine (b) epithelial thickness of OVX rats. Oestrogen = oestrogen treated rats; FA 150 = 150 mg/kg of FA extract treated rats; FA 300 = 300 mg/kg FA extract treated rats. (*p<0. 05), (**p<0.01).

days) resulted in a significant (p<0.01) reduction of serum triglyceride levels in all treatment groups compared to controls. Control animals on the other hand showed time dependent increase in serum triglyceride levels (Figure 3).

Serum total cholesterol

Like triglycerides, total serum cholesterol levels were much higher in OVX rats compared to non-OVX female rats from the baseline levels of 0.77±0.03 to 1.10±0.072 g/L (43%) 12 weeks post ovariectomy. Oestrogen treatment significantly decreased total serum cholesterol levels by 29.4% (p<0.05) and 36.7% (p<0.01) after the 14 and 28 days treatments periods, respectively. *F. adolfi friderici* 150 mg/kg significantly (p<0.05) reduced serum triglyceride levels while 300 mg/kg *F. adolfi friderici* extract had a weak effect on serum total cholesterol levels after 28 days of treatment (Figure 4).

Serum HDL-cholesterol

After the 14-day treatment period, serum HDL-cholesterol level increased significantly in both oestrogen treated (0.84±0.1, p<0.05) and *F. adolfi friderici* treated groups (*F. adolfi friderici* 150 mg/kg: 0.78±0.01; p<0.01; *F. adolfi friderici* 300 mg/kg: 0.80±0.11; p<0.05). However, after 28 days of treatment only the *F. adolfi friderici* 300 mg/kg treated rats had significantly (0.98±0.09; p<0.05) increased plasma HDL-cholesterol level (Figure 5).

Effect of treatment on bone structure

In addition to change in bone structure, OVX also significantly (11%, p<0.05) decreased relative bone mass. Histological analyses three months after oophorectomy revealed significant thinning of cortical bone and many resorption pits in untreated OVX rats (Figure 6). Both oestrogen-treated and *F. adolfi friderici* - treated OVX rats had thicker cortical bone and no visible resorption pits.

DISCUSSION

In the present study, the ethanolic extract of *F. adolfi friderici* was tested for its oestrogenic properties in OVX rats.

Ovariectomy resulted in a change in uterine smear cytology with a prevalence of parabasal cells which are characteristic of the transition from dioestrus to proestrus in rats. *F. adolfi friderici* treatment resulted in the presence of a few superficial cells while smears from oestrogen treated rats had both intermediate and superficial cells as well, indicating that treated animals were in the estrus phase. Superficial cells are found only in early estrus while both superficial and intermediate cells are characteristic of estrus. Although, the number of

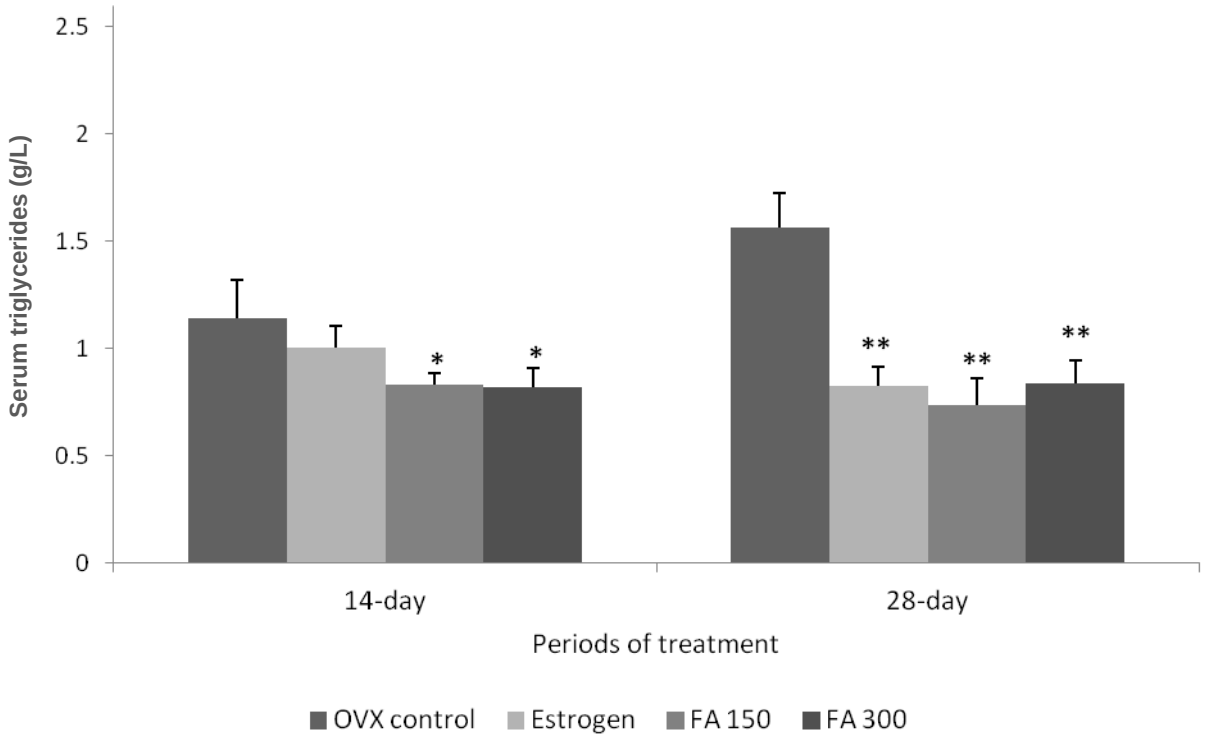

Figure 3. Serum triglyceride levels after 14 and 28 days of treatment respectively. OVX: ovariectomized; FA 150 = 150 mg/kg of FA extract treated rats; FA 300 = 300 mg/kg FA extract treated rats. (*p<0. 05), (**p<0.01).

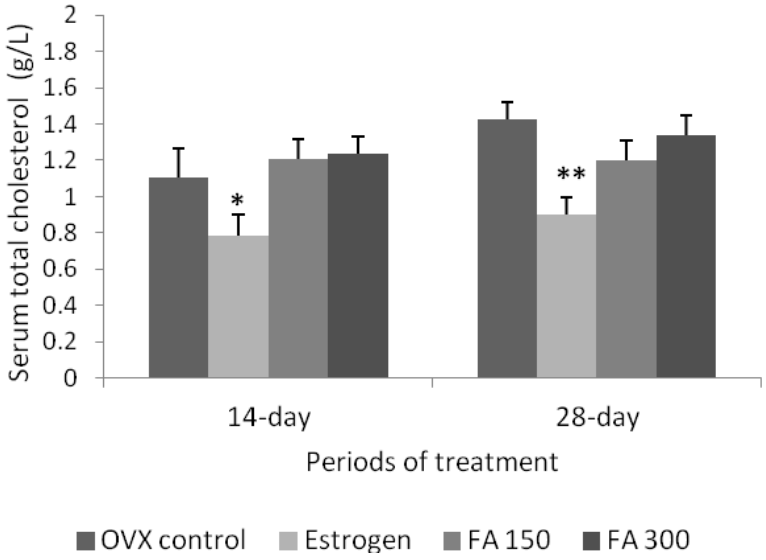

Figure 4. Serum total cholesterol levels after 14 and 28 days of treatment respectively. OVX: ovariectomized; FA 150 = 150 mg/kg of FA extract treated rats; FA 300 = 300 mg/kg FA extract treated rats. (*p<0. 05), (**p<0.01).

superficial cells in *F. adolfi friderici* treated animals was very low yet the current results indicate that these animals were in early estrus, while oestrogen treated animals were in estrus. The estrus-like phase shows that *F. adolfi friderici* extracts have a weak duration dependence effect on vaginal smear cytology (Goswami

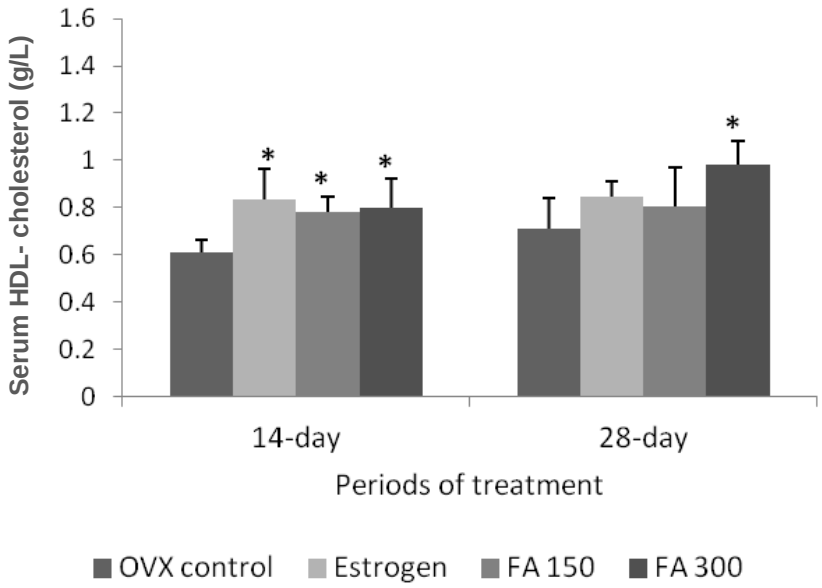

Figure 5. Serum HDL-cholesterol levels after 14 and 28 days of treatment respectively. OVX: ovariectomized; FA 150 = 150 mg/kg of FA extract treated rats; FA 300 = 300 mg/kg FA extract treated rats. (*p<0. 05).

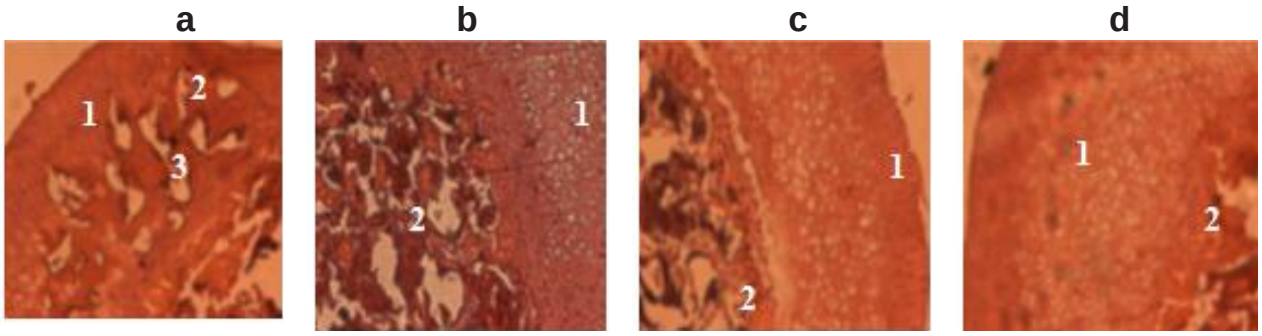

Figure 6. Bone architecture of control and treated OVX rats at the end of 94 days treatment period (a) OVX control, (b) Oestogen-treated OVX rats, (c) FA 150-treated OVX rats, (d) FA 300-treated OVX rats. 1 = cortical bone; 2 = trabecular bone; 3 = resorption pits.

et al., 2008). Cornification of the vaginal epithelium is one of the methods used to measure the oestrogenic effects of a drug (Diel et al., 2002; Laws et al., 2000; Jefferson et al., 2002). Indeed Kim et al. (2006) reported that oestrogen enhanced vaginal vascularization which according to Rockwell et al. (2002) became more permeable to numerous growth factors responsible for angiogenesis reducing vaginal wall thinning. Although, the vaginal epithelium and endometrium have similar oestrogen receptors, *F. adolfi friderici* unlike oestrogen did not show significant uterotrophic effects suggesting that *F. adolfi friderici* may contain secondary metabolites that may display tissue-specific oestrogenic or anti-oestrogenic properties. Oestrogen is known to cause endometrial hyperplasia (Hampton et al., 2005) an effect which has been associated with increased risk of uterine cancers.

At the end of the 14 days of treatment, *F. adolfi friderici* (both doses) significantly lowered serum triglycerides levels. Oestrogen treated rats showed a significant decrease in triglyceride levels only at the end of the second phase of treatment. Other studies have either shown an increase in serum triglyceride levels or a negative association between serum triglyceride levels and serum oestrogen levels in menopause (Cho et al., 2011). Our study however showed a time dependent lowering of triglyceride levels in OVX rats. Importantly, hypertriglyceridemia is a known risk factor for development of atherosclerosis in postmenopausal women. Prior to menopause or OVX the risk for atherosclerosis is reduced by oestrogen which contributes to the prevention of cardiovascular diseases by ensuring healthy lipid

profiles (Shearer et al., 2000). Indeed several studies have associated high serum triglyceride levels with increased risk of atherosclerosis and consequently heart diseases and stroke (Bansal et al., 2007; Bang et al., 2008).

Oestrogen significantly decreased total cholesterol levels after both 14 and 28 days of treatment. The lower dose of *F. adolfi friderici* however, significantly decreased total cholesterol levels only after 28 days of treatment while the higher dose failed to decrease serum levels of total cholesterol. Total cholesterol levels tend to increase in post menopausal women while hormone replacement therapy decreases it (Lye et al., 2009). Aging and consequently menopause adversely affect lipid profiles in women, total cholesterol, LDL and triglyceride levels increase with age while HDL levels tend to decrease (Kolovou and Bilianou, 2008). Total cholesterol is a measure of all the cholesterols and triglycerides. High cholesterol levels indicate an increased risk for heart disease, high blood pressure and stroke (Halperin et al., 2006; Paynter et al., 2011; Bowman et al., 2003). The cholesterol lowering effects of *F. adolfi friderici* were much weaker compared to oestrogen's; these effects became significant after 28 days of treatment indicating the need for prolonged use of the extract to derive its cholesterol lowering benefits.

Serum HDL-cholesterol levels were significantly increased in all treatment groups after 14 days of treatment; though by the 28th day it was only significantly higher in oestrogen treated animals. Bhagya et al. (2011) showed that serum HDL-cholesterol levels decrease significantly with menopause possibly due to the decrease in oestrogen levels associated with menopause.

Treatment with *F. adolfi friderici* improved on bone tissue microarchitecture, restoring both cortical and compact bone structure suggesting that *F. adolfi friderici* may stimulate bone formation. Furthermore, ovariectomy resulted in increased bone turn over with resorption exceeding formation. This imbalance is known to lead to progressive loss in bone mass and eventually osteoporosis (Bolognese et al., 2009). Our results suggest that prolonged treatment with *F. adolfi friderici* may not only improve bone microarchitecture, but may also restore normal bone mineralization by inhibiting the activity of osteocalsts and all the cytokines involved with bone resorption thus correcting the balance between bone resorption and formation.

Conclusion

F. adolfi friderici unlike oestrogen induced vaginal epithelial thickening while having no effect on endometrium. It also prevented menopause associated high lipid profiles and bone resorption indicating that *F. adolfi friderici* may be useful in preventing some of the symptoms of menopause.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

OVX, Ovariectomized/ovariectomy; **FA,** ethanolic extract of *Fernandoa adolfi friderici*.

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