Author's response to reviews

Title: Nigella Sativa reverses osteoporosis in ovariectomized rats

Authors:

Ansam A Seif (ansamseif@yahoo.com)

Version:2 Date:3 November 2013

Author's response to reviews:

Dear Dr Tom

These are the responses to the Editorial and the Reviewers’ comments. Thank you very much

Best Regards

Ansam

Responses to Editorial comments:

1- This manuscript is not under consideration at any type of government publication, and that statement is just a routine one that I include in the cover letter.

2- The nigella powder capsules were purchased from Bio Extracts Company (Baraka) Sri Lanka through their website (www.baraka.lk, www.bioextracts.lk). The active ingredient is Pure Black Seed Powder. Baraka Black Seed Capsules is approved by the Formulary Committee for Ayurvedic Medicine, Department of Ayurveda, Ministry of Health & Indigenous Medicine, Sri Lanka under Approval No. 02/01/PB/09/223

3- Authors’ Contributions: added; please refer to manuscript

Responses to Reviewer 1 report:

1- Title: changed, please refer to manuscript

2- Abstract: changes made, please refer to manuscript

3- Methods

a) The nigella powder capsules were purchased from Bio Extracts company (Baraka) Sri Lanka
through their website (www.baraka.lk, www.bioextracts.lk). The active ingredient is Pure Black Seed Powder.

Baraka Black Seed Capsules is approved by the Formulary Committee for Ayurvedic Medicine, Department of Ayurveda, Ministry of Health & Indigenous Medicine, Sri Lanka under Approval No. 02/01/PB/09/223

- b) I don’t have the uteri of these animals anymore, but osteoporotic changes were confirmed in the present study both biochemically and histologically which ensures the success of the ovariectomy procedure. Moreover, systemic osteoporosis has been proved by many studies to develop only by a bilateral ovariectomy

Kalu stated that

An animal model of postmenopausal bone loss can be defined as a living animal in which spontaneous or induced bone loss due to ovarian hormone deficiency can be studied, and in which the characteristics of the bone loss and its sequelae resemble those found in postmenopausal women in one or more respects. Although in comparison to humans, the skeletal mass of rats remains stable for a protracted period during their lifespan, rats can be ovariectomized to make them sex-hormone deficient, and to stimulate the accelerated loss of bone that occurs in women following menopause. Ovariectomy induced bone loss in the rat and postmenopausal bone loss share many similar characteristics. These include: increased rate of bone turnover with resorption exceeding formation; and initial rapid phase of bone loss followed by a much slower phase; greater loss of cancellous than cortical bone; decreased intestinal absorption of calcium; some protection against bone loss by obesity; and similar skeletal response to therapy with estrogen, tamoxifen, bisphosphonates, parathyroid hormone, calcitonin and exercise. These wide-ranging similarities are strong evidence that the ovariectomized rat bone loss model is suitable for studying problems that are relevant to postmenopausal bone loss.


Hidaka et al. used the mature ovariectomized rat model and stated that in order to develop bone loss in ovariectomized rats, all animals were maintained for 7 week after ovariectomy.

Hidaka S, Okamoto Y, Uchiyama S, Nakatsuma A, Hashimoto K, Ohnishi ST, Yamaguchi M.

Fathilah et al. used the mature ovariectomized rat model for 8 weeks.


Stimpel et al. reported that osteoporotic changes in the bones of the skeleton were confirmed histologically in all rats eight weeks after ovariectomy and were expressed by the thinning of bone trabeculae and increasing intertrabecular space, also observed in histological preparations microfractures of bone trabeculae.


c- ALP and NTx were chosen in the present study as representatives of bone formation and resorption because:

Alkaline phosphatase is an inexpensive method of checking for osteoblastic activity and has been numerously used as a marker of bone formation. Not only is it easy and inexpensive to test for alkaline phosphatase, but the routine nature of the testing may allow for earlier detection of increased bone activity.

Cross-linked N-telopeptides of type I collagen (NTx) are generally reliable markers of bone resorption because they are stable end products of bone resorption in serum and urine, and thus offer a valuable tool to monitor bone metabolism.

d- Bone morphometric analysis was performed.

Bone morphology parameters were analysed by Dr. Safaa Shaker, Assistant Professor of Histology, Faculty of medicine, Ain Shams University, who was blinded to the study.

e- Abbreviations have been stated in detail and countries stated; please refer to manuscript.

4- Results
Changes made; please refer to manuscript

5- Figures
Figure legends: checked

6- Discussion
- Grammar: re-checked
- Plasma tumor necrosis factor-# (TNF-#), and plasma interleukin-6 (IL-6) have been added; please refer to manuscript.

Responses to Reviewer 2 report:

1- To the best of our knowledge, the dose of nigella sativa used in the present study has not shown toxic effects in previous studies.

a) Ali and Blunden stated that the seeds are characterized by a very low degree of toxicity. Administration of either the seed extract or its oil has been shown not to induce significant adverse effects on liver or kidney functions. It would appear that the beneficial effects of the use of the seeds and thymoquinone might be related to their cytoprotective and antioxidant actions, and to their effect on some mediators of inflammation.


b) It was also reported that nigella sativa extracts are relatively nontoxic in the acute toxicity test, but the possibility of hepatic damage with its aqueous extract should be considered, that is in high doses as 6, 9, 14 and 21 g/kg.


c) Dollah et al. performed a study to determine the toxic effect of Nigella sativa powder on the liver function which was evaluated by measuring liver enzymes and through histopathological examination of liver tissue. The study showed that supplementation of Nigella sativa up to the dose of 1 g/kg supplemented for a period of 28 days resulted in no changes in liver enzymes level and did not cause any toxicity effect on the liver function


d) Also, Zaoui et al. reported that the low toxicity of Nigella sativa fixed oil, evidenced by high LD50 values, key hepatic enzyme stability and organ integrity, suggests a wide margin of safety for therapeutic doses of Nigella sativa fixed oil.


2- The nigella powder capsules were purchased from Bio Extracts company (Baraka) Sri Lanka through their website (www.baraka.lk, www.bioextracts.lk ). The active ingredient
is Pure Black Seed Powder.
Baraka Black Seed Capsules is approved by the Formulary Committee for Ayurvedic Medicine, Department of Ayurveda, Ministry of Health & Indigenous Medicine, Sri Lanka under Approval No. 02/01/PB/09/223

3- Tissues of animals used in the present study are not available anymore, so BMD can be done in further studies. Bone morphometric analysis in addition to histological and biochemical studies done in the present work could be quite sufficient to evaluate osteoporotic changes, and have been used in previous variable studies to evaluate osteoporotic changes.

4- I don’t have the uteri of these animals anymore, so the estrogenicity of Nigella Sativa can be done in further studies. This topic has been addressed in previous studies:

a) Keshri et al. stated that concerning the estrogenic activity of nigella sativa: When administered orally to ovariectomized immature female rats once daily for three consecutive days, 2 g/kg dose of the hexane extract of the seeds of N. sativa caused only mild uterotrophic effect, comparable almost to 0.002 mg/kg dose of 17 oc-ethinyl estradiol, but was devoid of any estrogenic activity. The reported lack of antiovulatory activity in the seed extract of this plant confirms absence of significant estrogenic activity in them.

G. Keshri, M. M. Singh, V. Lakshmr• and V. P. Kambor post-coital contraceptive efficacy of the seeds of nigella sativa in rats indian j physiol phannaco11995; 39(1) : 59-62

b) Parhizkar et al. suggested that N. sativa possesses estrogenic function in the ovariectomized rat model which can be helpful in managing menopausal symptoms as an alternative for Hormone Replacement Therapy. Furthermore the recovery of rats on vaginal cytology after receiving the synthetic estrogen, conjugated equine estrogen, is also slower than the feeding of N. sativa. This might be taken as an advantage of using N. sativa for hormone replacement therapy compared to the synthetic estrogens when the short-term effect is needed. These estrogenic activities of N. sativa could be attributed to the unsaturated fatty acid contents, which are proven to possess estrogenic effects in animals (Liu et al., 2004; Hu et al., 2007; Suzuki et al., 2008), in man and in cell cultures (Banu et al., 2006).

Saadat Parhizkar, Latiffah Abdul Latiff, Sabariah Abdul Rahman, Mohammad Aziz

c) Parhizkar and Latiff also reported that Linoleic acid (which is an omega-6 fatty acid richly found in nigella sativa) showed the beneficial effects on OVX rats’ reproductive performance (by Improvement of Vaginal Cornification of Ovariectomized Rats) thereby indicating its beneficial role in the treatment of the postmenopausal symptoms.

Saadat Parhizkar, Latiffah A Latiff Supplementary Health Benefits of Linoleic Acid by Improvement of Vaginal Cornification of Ovariectomized Rats Advanced Pharmaceutical Bulletin, 2013, 3(1), 31-36

5- Plasma tumor necrosis factor-# (TNF-#), and plasma interleukin-6 (IL-6) have been added; please refer to manuscript.

6- This can be done in further studies

7- Comparing the ovariectomized group to the sham-operated group is important to ensure osteoporotic changes and comparing the nigella sativa treated group with the sham-operated group is important to show how much Nigella Sativa treatment was effective in reversing osteoporotic changes seen in the ovariectomized group. All 3 groups have to be compared with respect to each other to show the inter-related significant changes between them.

8- Experimental processes have been added to the Methods section; please refer to manuscript.

9- Plasma Malondialdehyde (MDA) levels, an important measure of lipid peroxidation, were measured in the present study and were found to be significantly increased in OVX rats compared to both SHAM and OVX-NS rats.

10- Recently, the importance of preventive medicine has been gradually recognized in the field of orthopaedic surgery with a concept that peak bone mass should be increased in childhood as much as possible for the prevention of osteoporosis. This has been reported by Ohtani J, Hernandez RA, Sunagawa H, Fujita T, Kawata T, Kaku M, Motokawa M, Tsuka N, Koseki H, Matsuda Y, Hayashi H, Abedini S, Tanne K. A newly developed snack effective for enhancing bone volume. Nutr J. 2009;3:8–30.

Moreover, Hidaka et al. used the mature ovariectomized rat model and stated that in order to develop bone loss in ovariectomized rats, all animals were maintained for 7 week after ovariectomy.

Hidaka S, Okamoto Y, Uchiyama S, Nakatsuma A, Hashimoto K, Ohnishi ST, Yamaguchi M.

Fathilah et al. used the mature ovariectomized rat model for 8 weeks.


Furthermore, Das reported that IL-1 and TNF-α were increased in healthy premenopausal women who underwent ovariectomy and reached the highest levels 8 weeks after ovariectomy, and these changes were associated with indices of bone resorption.


Under such background, Ovariectomized (OVX) rats have been supplemented with nigella sativa four weeks before ovariectomy aiming to evaluate the efficacy of nigella sativa supplementation starting before menopause. Supplementation continued for 8 weeks after ovariectomy to ensure the development of osteoporotic signs in OVX rats as shown in previous studies.

11- Although in osteoporosis, cancellous bone is more severely affected than cortical bone, yet cortical bone still shows affection.

Danielsen et al. showed that ovariectomy induces significant decrease in cortical bone.


Seeman mentioned that estrogen deficiency at menopause led to more bone resorption and less bone formation causing cortical thinning.


Our results show that ovariectomy (OVX) resulted in significant decrease in cortical bone thickness, which was nearly reversed by nigella sativa supplementation compared to SHAM group. NS supplementation in OVX-NS was effective in preventing hypocalcemia caused by ovariectomy in OVX rats, which could be attributed to its high content of unsaturated fatty acids. Moreover, it has been shown to possess antioxidant and anti-inflammatory properties, which might further add to its anti-osteoporotic effect. Taken together, nigella sativa supplementation reversed osteoporotic cortical bone changes caused by ovariectomy.