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Comparative Effect of Atorvastatin and *Trigonella foenum graecum* L. Seeds in the Postmenopausal Hyperlipidemia

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Abstract

Context: *Trigonella foenum graecum* L. (Fabaceae) seeds have been extensively used in Ayurveda and Unani medicine. It has been used as a traditional medicine for a household remedy against various human ailments. The seeds have been shown to possess hypoglycemic, hypocholesterolaemic and antioxidant activity. Objective: The present study is to evaluate whether ovariectomised (OVX) Wistar rats could form an experimental model of postmenopausal hyperlipidemia and to evaluate the antihyperlipidemic potential of Atorvastatin (AT) and Ethyl acetate extract of *Trigonella foenum graecum* L. Seeds (ET) in OVX Wistar rats and toxicity associated with it. Methods: Ovariectomy was performed to mimic the postmenopausal hyperlipidemic condition in Wistar rats. The effects of AT and ET on body weight, weight of uterus and the levels of total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoprotein (VLDL) were also evaluated. Results: The rats in OVX control group showed 51% weight gain when compared with vehicle control group. The serum TC, TG, LDL, VLDL of OVX control group was found to be increased significantly; serum HDL level was reduced and atrophy of uterus was observed in comparison with vehicle control group. The ET showed the significant ($P<0.05$, $P<0.01$) antihyperlipidemic potency when compared with AT & proportional antihyperlipidemic potency in comparison with Estradiol benzoate (EB). Conclusion: These findings confirm the bilateral OVX Wistar rats as a model of postmenopausal hypercholesterolemia. The significant antihyperlipidemic activity of ET thus lends pharmacological credence to the suggested use of the plant as a safe natural remedy in the treatment of postmenopausal hyperlipidemia.

Keywords: Postmenopausal hyperlipidemia; ovariectomy; phytoestrogens

Introduction

Menopause, whether natural or surgical, is associated with elevations in circulating total and LDL cholesterol concentrations, placing postmenopausal women at greater risk for coronary heart disease (CHD) [1]. These changes are a consequence of reductions in the levels of circulating estrogens, which is the basis for estrogen replacement therapy (ERT). ERT reduces the risk of CHD in part through the modulation of serum cholesterol. However, ERT and other cholesterol-lowering pharmacological agents may be accompanied by side effects [2] and therefore are recommended only for women without known contraindications. Moreover, many women chose not to comply with a recommendation for ERT because of safety concerns. Therefore, other means that present no known side effects for the treatment of postmenopausal hypercholesterolemia are preferred.

Phytoestrogens are naturally occurring plant chemicals that can produce an estrogen-like effect in the body, used as a natural alternative to hormone replacement therapy and to reduce menopausal symptoms. They are not chemically the same as the estrogens made in the body, but when digested and absorbed they can act somewhat as estrogen in the body [3].

Trigonella foenum graecum L. seeds have been exercised to reduce the hyperlipidemia which forms a major risk for cardiovascular disease. The seeds have been proved for their potential estrogenic activities [4]. Its main components are flavonoids and saponins. Recent studies have found that both components have significant hypocholesterolaemic effects [5]. Atorvastatin is the most efficacious of the currently available HMG-CoA reductase inhibitors in terms of its LDL cholesterol lowering effect. In addition, it has a modest triglyceride lowering action.

In the present study ovariectomy of the female Wistar rats have been resulted in reduction of estrogen level in their body, which might lead to the body weight gains, increase in feed consumption, imbalance of lipoprotein metabolism and atrophy of uterine. Therefore, the OVX rats can be used as a model to reflect the pathological changes in perimenopausal or postmenopausal women.

Methods

Plant Material and Extraction

Seeds of *Trigonella foenum graecum* L. were procured from the local market in Gujarat, India and authenticated by Dr. Minoo Parabia [6]. Ethyl acetate extract of *Trigonella foenum -graecum* L. (Fenugreek) seeds were obtained by soxhlet extraction of *Trigonella foenum graecum* L. seeds with ethyl acetate (98%) for 12 hours and concentrated using rotary vacuum evaporator at 40-60 C. The concentrated extract was stored in refrigerator at 2-8 C. Qualitative phytochemical evaluation of ET was performed [7].

Animals

The animal experiment protocol was approved by Institutional Animal Ethics Committee, Jai Research Foundation, Vapi – Gujarat, India and was performed in accordance with guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Thirty adult female Wistar rats (6-8 weeks old) were randomized in five treatment groups of six animals each. Animals were housed at 23 ± 2 C and relative humidity 60 ± 5% with 12 h light-dark cycle. Food (standard rodent pellet diet) and water was provided *ad libitum*. Four groups were bilaterally ovariectomized while one group was kept as vehicle control. Ovariectomy was performed under ketamine and xylazine anaesthesia (80 mg/kg + 10 mg/kg; i.p.) according to the method described in OECD 440 Guidelines [7]. Animals were housed individually for two weeks after the surgery and later on in a group of two. Body weight of the animals was recorded weekly.

Study Design

Vehicle control and OVX control animals were administered orally 0.5% w/v sodium Carboxy methyl cellulose (sodium CMC) (SD Fine - Chem. Ltd., India) which was used as vehicle. The other three OVX groups were administered 17-β-Estradiol 3-benzoate (Sigma) equivalent to 0.15 mg/kg of β-estradiol, Atorva 5 Tablets (Zydus Healthcare, India) equivalent to 10 mg/kg of Atorvastatin and 1000 mg/kg of ET. Dose of ET was decided based on the results of the preliminary Acute Oral Toxicity study as described in OECD 423 Guidelines [8]. Suspensions of β-estradiol benzoate, AT and ET were prepared in 0.5% w/v of sodium CMC. Drug treatments were started after 2 weeks of ovariectomy and were continued for 60 days. Blood was collected from orbital plexus at the interval of 30 days for estimation of biochemical parameters. On the day 60, animals were sacrificed by carbon dioxide asphyxiation. All animals were subjected to gross necropsy. The uteri were isolated, relative weights of uterine tissue was recorded and were subjected to histopathological examination.

Biochemical Analysis

Serum TC, TG and HDL levels were measured by using commercially available estimation kits (Span Diagnostics Ltd., India) based on enzymatic assay method and serum LDL and VLDL levels were measured by Friedewald Formula [9].

Statistical Analysis

The raw data were processed by the Department of Bio-statistics and Systems Information, Jai Research Foundation to get group means and standard deviations with significance between the control and treated groups using in-house developed statistical software. Appropriate statistical methods (Student *t*-test & One way ANOVA followed by Dunnett's multiple comparisons test) were employed to assess the significance among different groups with significance level of $P<0.05$.

Results

The percentage yield of ET was found to be 10% w/w of coarse powder of seeds of *Trigonella foenum graecum* L. Preliminary phytochemical investigation revealed that ET contains alkaloids, flavonoids, saponin glycosides, protein, amino acids and mucilage. The maximum tolerated dose (MTD) of ET was found to be 5000 mg/kg according to OECD 423 Guidelines.

Effect on percentage body weight of OVX rats

Statistical significant increase in percentage body weight of female rats of OVX control group ($P<0.05$) on day 56 and 60 was observed when compared with vehicle control group (Figure 1). No significant results were obtained in percentage body weight of female rats of OVX + EB (0.15 mg/kg), OVX + AT (10 mg/kg) and OVX + ET (1000 mg/kg) groups when compared with OVX control group. The feed intake of OVX control rats was also significantly increased in comparison of vehicle control group (Data not shown).

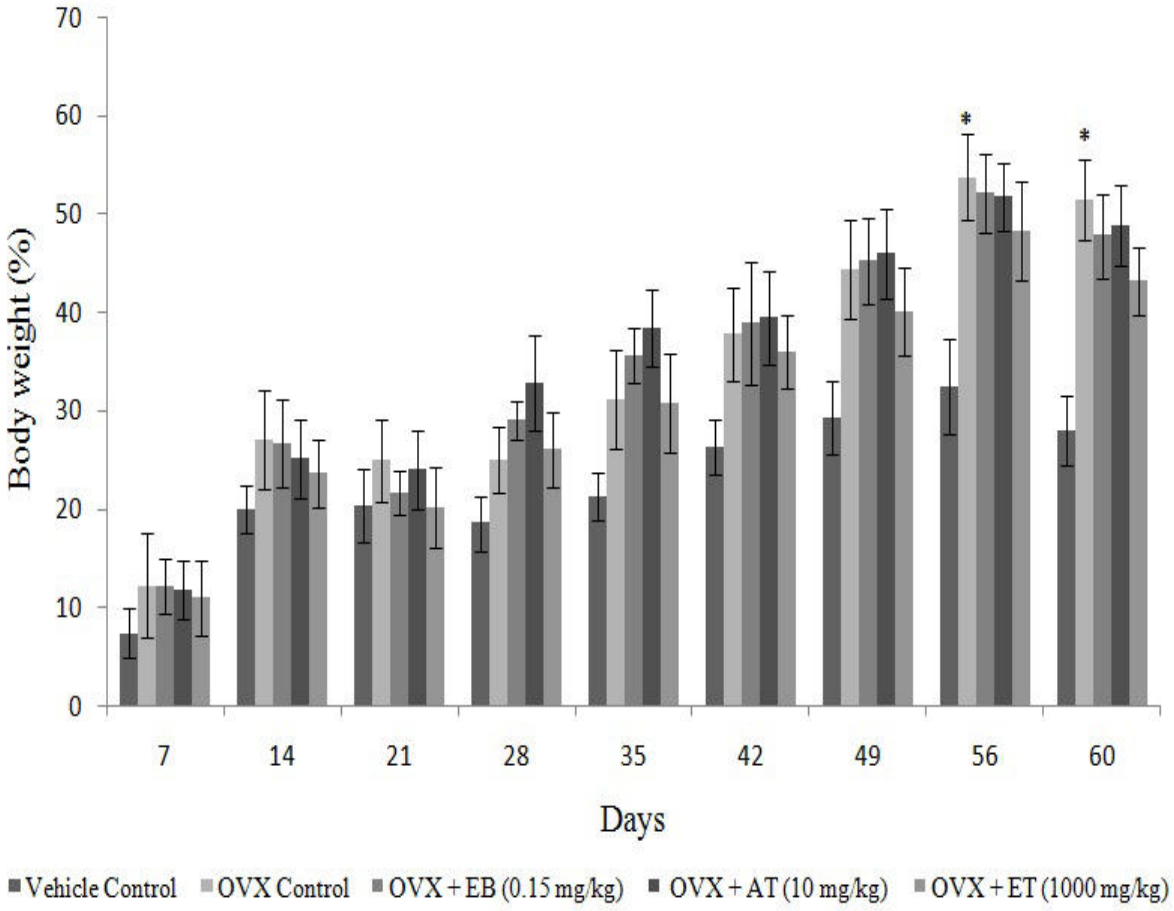

Figure 1. Effect of AT and ET on percentage body weight of OVX rats. Values are expressed as Mean ± SD (n=6).
* P<0.05 *vs.* vehicle control group.

Effect on biochemical parameters of OVX rats

Serum TC, TG, LDL and VLDL Levels

Significant increase (P<0.01) in serum TC and LDL levels in female rats of OVX control group on day 30 and 60 was observed when compared with vehicle control. There was significant decrease (P<0.01) in serum TC and LDL levels in female rats of OVX + EB (0.15 mg/kg) group, OVX + AT (10 mg/kg) group and OVX + ET (1000 mg/kg) group on day 30 and 60 was observed when compared with OVX control group, respectively (Figure 2 & 3).

On the day 60, statistically significant decrease (P<0.05) in TC level in female rats of OVX + AT (10 mg/kg) was observed when compared with OVX + EB (0.15 mg/kg) group (Figure 2).

Significant increase (P<0.01, P<0.05) in serum TG and VLDL level in female rats of OVX control group on day 30 and 60 was observed when compared with vehicle control, respectively (Figure 4 & 5). The initial raise in serum TG and VLDL cholesterol levels of OVX rat were reduced to 25%, 41% and 38% by the treatments of EB, AT and ET respectively.

Statistically significant decrease (P<0.01) in TG and VLDL levels in female rats of OVX + AT (10 mg/kg) on day 60 was observed when compared with OVX + EB (0.15 mg/kg) group and significant decrease (P<0.05) was observed in TG and VLDL levels in female rats of OVX + ET (1000 mg/kg) group on day 60 when compared with OVX + EB (0.15 mg/kg) group.

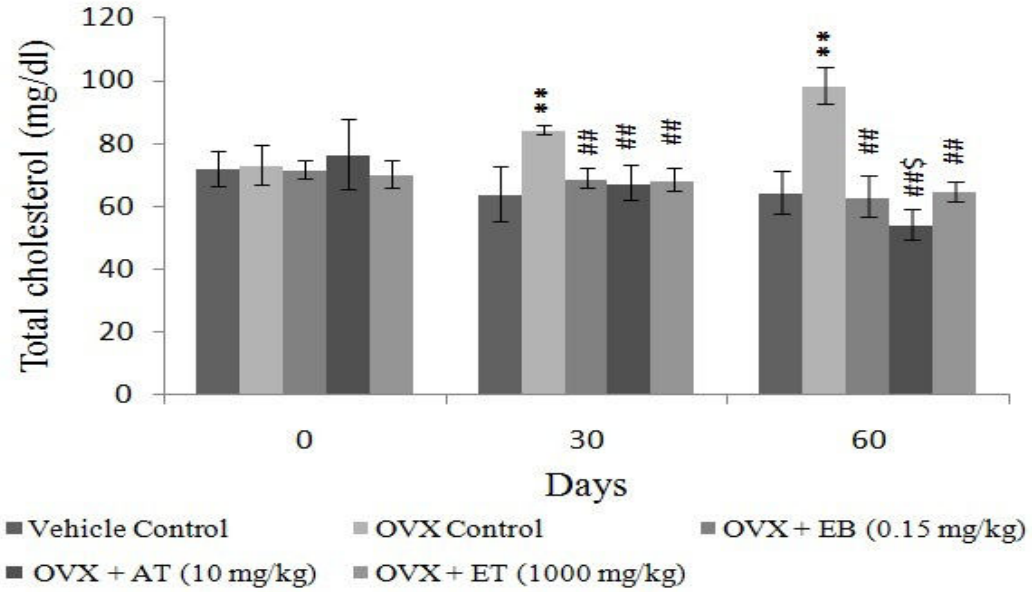

Figure 2. Effect of AT and ET on TC (mg/dl) of OVX rats. Values are expressed as Mean ± SD (n=6). **P<0.01 *vs.* Vehicle control; ##P<0.01 *vs.* OVX control; $P<0.05 *vs.* OVX + EB.

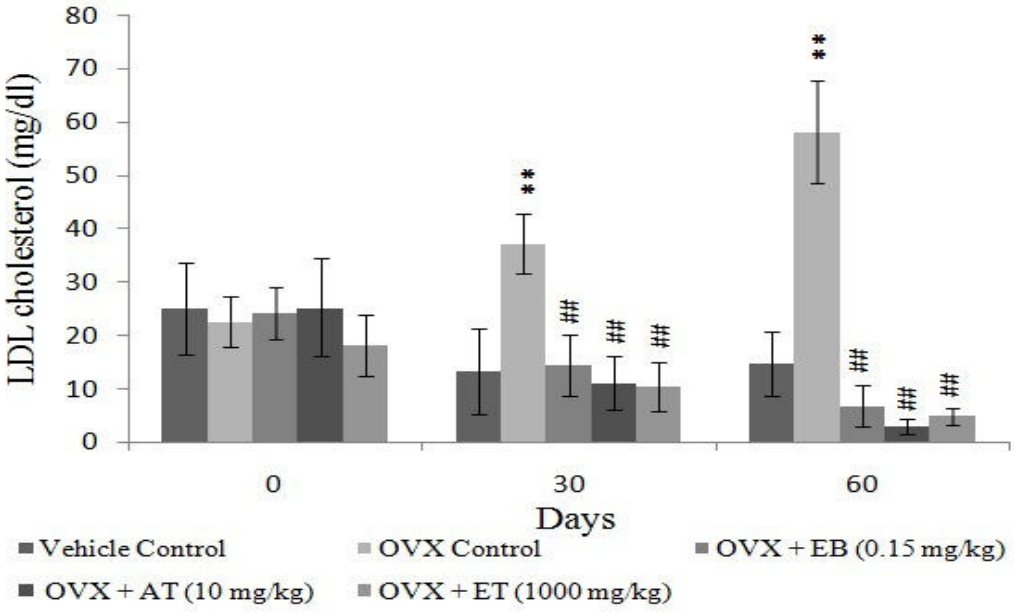

Figure 3. Effect of AT and ET on LDL (mg/dl) of OVX rats. Values are expressed as Mean ± SD (n=6). **P<0.01 *vs.* Vehicle control; ##P<0.01 *vs.* OVX control.

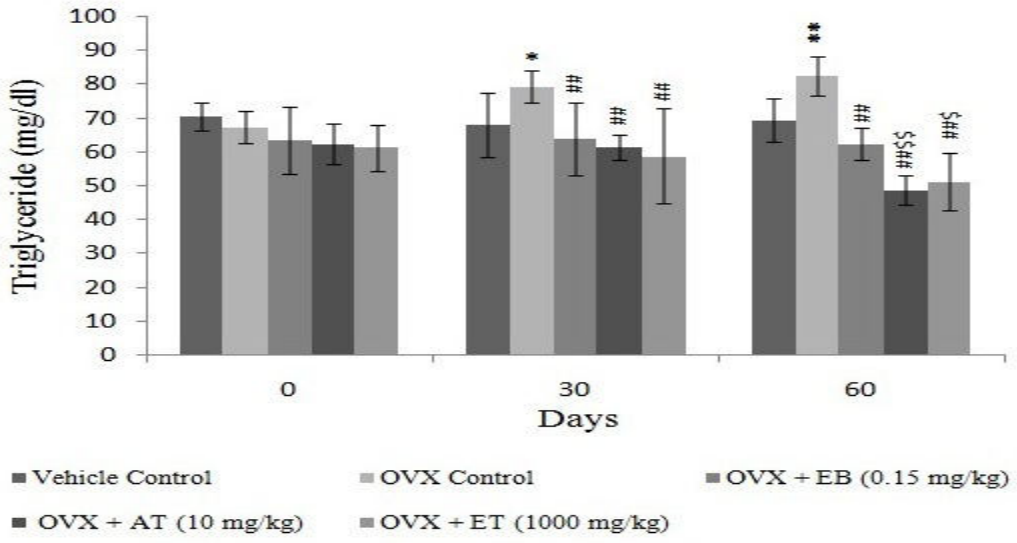

Figure 4. Effect of AT and ET on TG (mg/dl) of OVX rats. Values are expressed as Mean ± SD (n=6). * P<0.05, **P<0.01 *vs.* Vehicle control; #P<0.05, ##P<0.01 *vs.* OVX control; $P<0.05, $P<0.01 *vs.* OVX + EB.

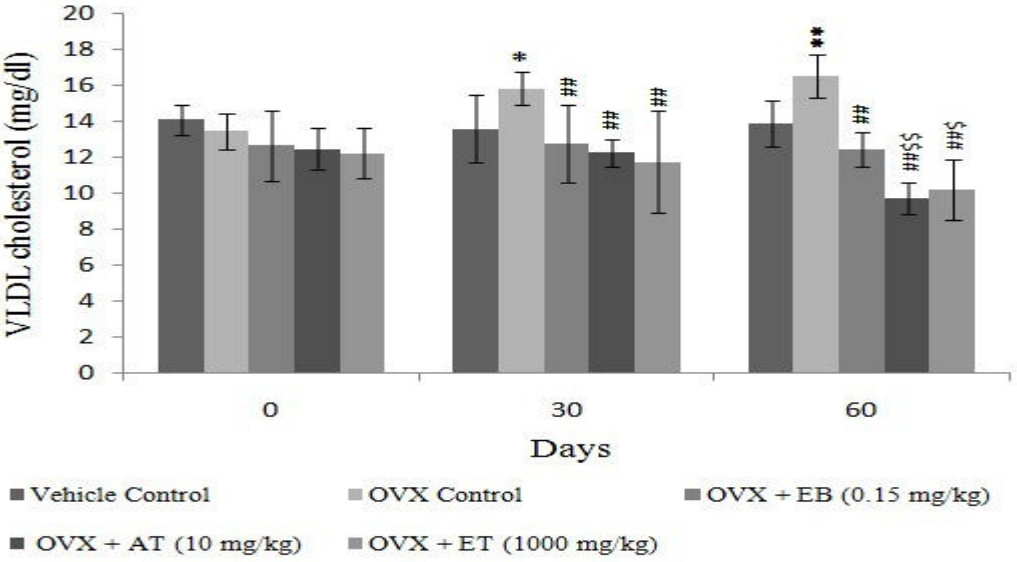

Figure 5. Effect of AT and ET on VLDL (mg/dl) of OVX rats. Values are expressed as Mean ± SD (n=6). * P<0.05, **P<0.01 *vs.* Vehicle control; #P<0.05, ##P<0.01 *vs.* OVX control; $P<0.05, $P<0.01 *vs.* OVX + EB.

Serum HDL levels

Significant decrease (P<0.01) in serum HDL cholesterol level in female rats of OVX control group on day 60 was observed when compared with vehicle control group. There was significant increase (P<0.01) in serum HDL cholesterol level in female rats of OVX + EB (0.15 mg/kg) group, OVX + AT (10 mg/kg) group and OVX + ET (1000 mg/kg) group on day 60 was observed when

compared with OVX control group. On day 30, there was significant increase (P<0.01) in serum HDL cholesterol level in female rats of OVX + AT (10 mg/kg) group & OVX + ET (1000 mg/kg) group was observed when compared with OVX control group (Figure 6).

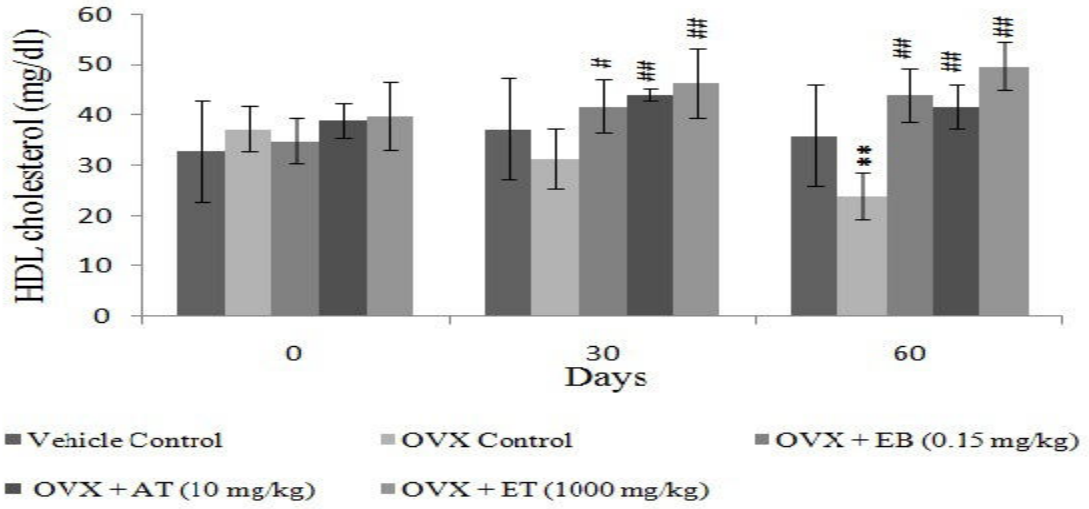

Figure 6. Effect of AT and ET on HDL (mg/dl) of OVX rats. Values are expressed as Mean ± SD (n=6). **P<0.01 *vs.* Vehicle control; #P<0.05, ##P<0.01 *vs.* OVX control.

Effect on relative weight of uterus in OVX rats

There was significant decrease (P<0.01) in relative weight of uterus in female rats of OVX control group when compared with vehicle control group. Treatment with the EB significantly increased (P<0.05) the relative weight of uterus when compared with OVX control group where as no significant change was observed in relative weight of uterus in female rats of OVX + AT (10 mg/kg) and OVX + ET was observed when compared with OVX control group (Figure 7).

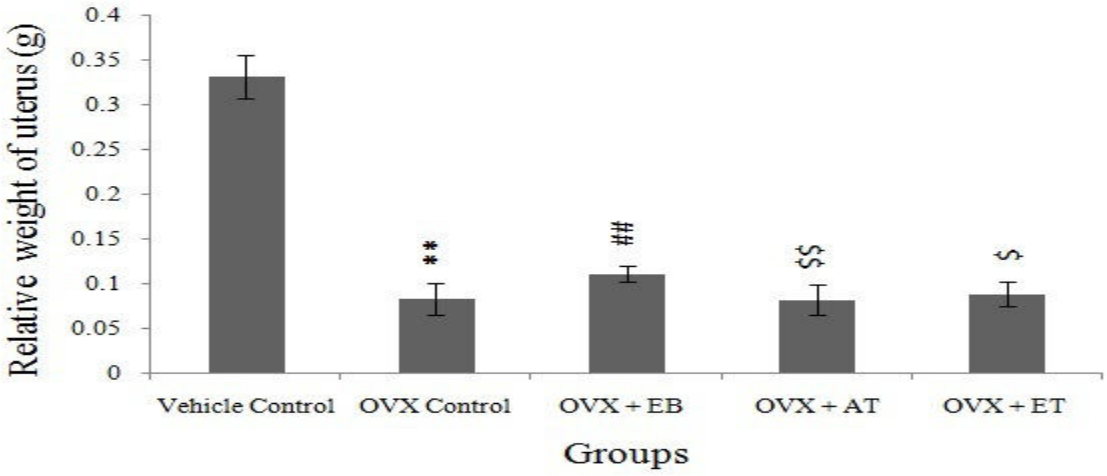

Figure 7. Effect of AT and ET on Relative weight of uterus (g) of OVX rats. Values are expressed as Mean ± SD (n=6). **P<0.01 *vs.* Vehicle control; ##P<0.01 *vs.* OVX control; $P<0.05, $$P<0.01 *vs.* OVX + EB.

Discussion and Conclusion

Most mammals maintain their reproductive capacity throughout their life and do not experience natural menopause. However; the menopausal state in animals can be induced by ovariectomy. This is comparable to the situation in women who undergo bilateral ovariectomy before menopause and prematurely experience the symptoms and side effects of menopause soon after surgery. The present study in ovariectomized rats provides the basis for an experimental model of menopause.

The effective induction of ovariectomy was confirmed by 51% increase in body weight when compared with vehicle control group. As expected, the significant weight gain most likely brought about by an increase in food intake [10]. Ovariectomy in the present experiment resulted in an increase in mesenteric and subcutaneous fat depots.

The present study confirms that ovariectomy increases the serum total cholesterol, triglycerides and other non – HDL cholesterol concentrations in Wistar rats, similar to observation made in postmenopausal women. Thus OVX rat is a good model for studying the effects of ovarian hormones on lipoprotein metabolism without the complications of existing endogenous hormones. Treatment with AT and ET have been reversed the ovariectomy induced increase in the serum total cholesterol, triglycerides and other non – HDL cholesterol concentrations in Wistar rats.

However, the mechanisms for these changes are not quite clear yet. The increased insulin concentration in OVX rats may accelerate the dephosphorylation effect of the hydroxyl methyl glutaryl CoA reductase (HMGCoA reductase), which is a rate-limiting enzyme of the composition of cholesterol. As a result, it accelerates the activity of the enzyme. Insulin could also induce the composition of the HMGCoA reductase directly to increase the composition of cholesterol. For the cholesterol metabolism *in vivo*, there are two transport pathways-the endogenous cholesterol transport and the reverse cholesterol transport. In the former, the cholesterol by exogenous absorption and synthesis in the liver is utilized when it is in the form of LDL-C, is combined with the receptors of the extra hepatic tissue, and the ligand combined with LDL receptors are mainly the apolipoprotein of apo B100 and apo E. In the latter, the extra hepatic cholesterol, in the form of HDL-C, is conveyed back to the liver and expelled out of the body after further metabolism.

Some experiments have shown that estrogen can enhance the activity of the LDL receptors and promote the endogenous transport so as to lower the TC levels. In conclusion, the high insulin level in OVX rats may lead to a high level of cholesterol concentration, while, the low level of estrogen concentration may result in the low level of LDL-R concentration and activity, both of which can cause an accumulation of LDL. The increased production and decreased consumption together make a high level of cholesterol concentration in the body, followed by the hypercholeste and adiposis hepatica [11].

Atorvastatin is a selective competitive inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase enzyme. In animal models, Atorvastatin leads to greater decrease in plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic low density lipoprotein (LDL) receptors on the cell surface to enhance uptake and catabolism of LDL; Atorvastatin also reduces LDL production and the number of LDL particles than other statins [12]. They also reduce plasma Triglycerides and increase HDL – cholesterol level.

Trigonella foenum graecum L. seeds contain biologically active components like alkaloids, steroidal saponins, fiber rich gum, and flavonoids etc. which do not directly interact with cholesterol. The flavonoids mainly kaempferol 3-O-glycoside, apigenin 7 – O – rutinoside and naringenin are present in ethyl acetate extract of fenugreek seeds. The presence of these flavonoids, especially naringenin revealed significant hypocholesterolaemic effects and antioxidant activity in ethyl acetate extract of fenugreek seeds [13].

Recent studies have further investigated the effect of naringenin on lipid and lipoprotein metabolism in HepG2 cells. Significant reductions in apoB secretion were observed after 24-hour incubation with naringenin. Naringenin has been reduced the assembling and secretion of apoB containing lipoproteins such as LDL and VLDL by direct inhibition of hepatic Acyl coenzyme A: cholesterol acyltransferase (ACAT) enzyme and due to 50% decrease in the mRNA for the microsomal triglyceride transfer protein (MTP), a protein known to be essential for the assembly and hepatic secretion of apoB-containing lipoproteins. Other studies demonstrated that fecal bile acid and cholesterol excretion are increased by fenugreek administration. This may be secondary to reaction between bile acid and fenugreek derived saponins causing the formation of micelles too large for the digestive tract to absorb. It is likely that both mechanisms contribute to the overall effect [14].

Histopathological evaluation of the uterine section revealed changes characterized by atrophy of uterus in ovariectomized rats (Figure 8a & 8b). Generally treatment with uterotrophic agents mainly estrogen increased endometrial response as indicated by proliferation of the endometrial glands, development of folds of the endometrial surface, dilation of lumen and increased vascularity [15].

In the present study, the treatment with estrogen has been produced the slight increase in the endometrial response might be indicated the dose dependent effect of estrogen. The treatment with AT and ET have unaltered the ovariectomy induced histomorphological changes, which revealed no positive influence on growth of uterine tissue. Profound studies are still needed to confirm its mechanisms.

In clinic, the commonly encountered side effects of Hormone Replacement Therapy (HRT) are edema (fluid retention), nausea, breast tenderness, and headache, increased growth of facial hair, dizziness, and hypophrodisia. More serious potential health risks to consider of HRT are breast cancer, uterine cancer, blood clotting, and liver and gallbladder diseases [16]. Today, selective estrogen receptor modulators (SERMs) have been used as an alternative approach to activate estrogen signaling pathways in a tissue-specific manner [17].

Phytoestrogens have a similar chemical structure to estrogen, and could bind to the estrogen receptor (ER)-beta. Once they bind each other, the ER is occupied, and the real estrogen cannot get in. Phytoestrogens then dispatch estrogen-like messages to the cells. Although it is a weak message, it is strong enough to produce some of the positive effects of estrogen but it is still too weak to stimulate the growth of cancer cells [18].When the estrogen levels are too high, this competition appears to reduce the effects of estrogen by replacing estrogen with the weaker phytoestrogen. When the estrogen levels are too low, it appears that phytoestrogen simulates the effects of estrogen and partially makes up for the deficiency [19].

These findings confirm the bilateral ovariectomised Wistar rats as a model of postmenopausal hypercholesterolemia. As a natural estrogen replacement, The ET showed significant antihyperlipidemic potency and did not exert any sign of toxicity in OVX rats. Further studies are required to be carried out to predict detailed mechanism of action of ethyl acetate extract of *Trigonella foenum graecum* L. seeds in order to reduce the risk of cardiovascular diseases in ovarian hormone deficiency.

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Declaration of interest

We declared there is no potential conflict of interest.

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