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Comparative effect of soy milk powder and estradiol on serum lipid profile of ovariectomized female Wistar rat

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Abstract

The decline in estrogen levels in post-menopausal women is known to cause adverse disruptions in lipid profile. To investigate potential interventions, this study examined the effects of soybean milk powder on ovariectomized rats' lipid profiles. Twenty adult female rats were divided into four groups (n =5): Group 1 was ovariectomized and supplemented with 3 mL of soybean milk containing 0.396mg of isoflavone given through the oral route, Group 2 was ovariectomized without supplementation, Group 3 received 0.2 mL of estradiol (a standard drug for the treatment of menopausal symptoms) intramuscularly (IM) after ovariectomy and Group 4 was sham operated and supplemented with 3 mL of drinking water (control). Standard laboratory procedures were employed to measure serum total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides (TG) over an 8-week period. The results indicated that ovariectomy significantly increased total cholesterol, LDL and TG levels ($p<0.05$), but no significant difference was observed in HDL levels ($p>0.05$) among the groups. Rats supplemented with soybean milk powder (Group 1) exhibited lower total cholesterol, LDL and TG levels compared to the un-supplemented ovariectomized group (Group 2). Group 1 and Group 3 (estrogen-treated) showed similar lipid profiles. Both soy milk powder and estradiol effectively reduced the increased TC, LDL and TG levels induced by ovariectomy. These findings suggest that soybean milk supplementation may hold promise as a potential strategy to mitigate adverse lipid profile changes in menopausal women. Further studies are warranted to explore the full potential and safety of this intervention.

Keywords: Cholesterol, Estrogen, Low lipid profile, Soflavone, Menopause, Ovariectomy

Introduction

Menopause is the cessation of monthly menstrual cycle in women. It occurs due to the loss of ovarian function and decreased estrogen production. There is a reduction in ovarian follicular reserve and estrogen production during this period (Santoro *et al*., 2021). Reduced estrogen levels after menopause result in structural, physiological and biochemical changes that affect the general health of post-menopausal women. This normal life transition is associated with short term vasomotor symptoms (hot flashes, mood swings, depression, nervousness and irritability), urogenital symptoms (recurrent vaginitis and dysuria) and long-term health conditions such as osteoporosis and cardiovascular diseases (Comparetto & Borruto, 2023). The incidence of cardiovascular disease is reportedly higher in post-menopausal women than in aged matched men and premenopausal women (Colpani *et al.,* 2014). It has been documented that post-menopausal women have more unfavourable lipid profiles than pre-menopausal women. There is an adverse reduction in high-density lipoprotein-cholesterol (HDL-C) levels and an increase in total cholesterol (TC), triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C). Home replacement therapy is the standard drug for the treatment of menopausal syndrome (Iorga *et al.,* 2017). The protective effects of estrogen against cardiovascular diseases are very obvious during the premenopausal period (Iorga *et al*., 2017). Estrogen therapy is very effective in improving the lipid profile of post-menopausal women (Gregersen *et al.,* 2019). Studies, however, have shown that estrogen therapy increases the risks of coronary heart disease, stroke, pulmonary embolism and breast cancer (Marco & Marco, 2014). Estrogen-like compounds in plants such as soy have been suggested as an alternative in the management of health problems emanating from estrogen deficiency (Cornwell *et al.,* 2004). According to the review conducted by Messina (2014), soy foods have a positive effect on the health of postmenopausal women. The health benefits were recorded in bone health, cardiovascular health and cancer prevention. He attributed these beneficial effects to the isoflavone content of soy foods. Phytoestrogen (isoflavones) has structural similarity with estrogen and hence can bind to estrogen receptors and influence cell proliferation and cell differentiation.

Nigeria is known to be a producer of soy bean where it is processed into different forms including soy milk powder. As soybean is readily available in Nigeria, it will be wise to evaluate the potential effects of soy milk powder on the lipid profile in ovariectomized female rats.

Materials and Methods

Animals and treatment

Twenty female rats aged 3 months old with average weight of 250±8.6 g, were randomly divided into 4 groups of 5 rats each (n = 5). The rats were fed pelleted feeds (Vital Feed®, produced by Grand Cereal Limited, Nigeria). They were reared on natural day light and were housed in a cage. Group 1 rats were ovariectomized and received soy milk as a supplement at a dose of 3 mL (containing 0.396mg of isoflavone) per rat per day, and was given orally using a gavage tube. The soybean used for the experiment was purchased from local market and was processed locally using frying and subsequent grinding methods. Group 2 rats were ovariectomized with no supplement. Group 3 rats were ovariectomized and received estradiol injection (Indiabulls Pharmaceutical, India) intramuscularly at a dosage of 0.8mg/kg serving as the control group. Group 4 rats were sham-operated and received 3ml of drinkable water which served as a positive control. Group 1 received soymilk supplementation once daily.

The whole experiment lasted for 8 weeks.

The soybeans used were processed by frying followed by coarse grinding to separate the chaff from the seed. The seed was finely grinded and used for feeding the rats. The isoflavone content in the soybean powder was determined using a spectrophotometer according to the method described by César *et al.* (2008). Total Isoflavone content was 40 mg/100 g of soy milk powder (Genistein = 19mg/100g and diadzein 12 mg/100 g). Soybean powder was dissolved in distilled water to produce 0.33g/mL soy milk solution used for the study. The soy milk solution contains 0.132 mg/ml of isoflavone.

Ethical approval

The work was carried out using the ethical standard as approved by the Faculty of Veterinary Medicine Institutional Animal Care and Use Committee (FVM-IACUC), University of Nigeria, Nsukka (UNN) with application reference number FVM-UNN-IACUC-2020-0447.

Ovariectomy of experimental rats

Animal preparation: The rats were positioned in dorsal recumbency. The furs were clipped with electric clipper from the xyphoid area to the pelvic brim. Chlohexidine® (4%) (AVA, Inc., USA) was used to disinfect the shaved area.

Anaesthesia**:** The rats were pre-medicated with 2% xylazine hydrochloride® (Bioveta, Czech Republic) at a dose of 10 mg/kg intramuscularly (IM) at the hamstring group of muscles, while anaesthesia was inducted with ketamine hydrochloride® 100 mg/mL (Laborate Pharmaceutical, India) at the dose of 50 mg/kg (Khajurial *et al*., 2012).

Surgical procedure: A small ventral midline laparatomy incision 0.4-0.6 cm was made with scalpel blade. After accessing the peritoneal cavity, the ovary and the associated fat were carefully located and exteriorized by gentle retraction. The ovarian vessels were ligated caudal to the ovary using chromic catgut (3/0) (Unigut® Unisur Lifecare, India). A second ligature was placed at the broad ligament of the ovary and a transection made at the cranial and caudal ends of the ovary. The procedure was repeated for the left ovary through the same incision.

The uterine horns were returned to the peritoneal cavity after the removal of the ovaries. The peritoneum and the muscle layers were closed with a simple continuous suture pattern using absorbable suture (chromic sutures-3/0) and the skin was closed with a horizontal mattress suture pattern with non-absorbable sutures (Unisil®-3/0 Unisur lifecare, India). Povidone iodine® (10%) (Qualitest Products Inc. USA) was applied on the area to disinfect the skin after suturing.

Post-operative care: After surgery, the rats were injected with 1mg/kg piroxicam (xirocam®, 20 mg/ml, Aden Healthcare, India) for pain management. The rats were housed individually in cages for a period of one week to allow recovery and then re-grouped in their home cages.

Determination of serum lipids

Blood samples were collected by venipuncture from the retro-bulbar vein into sterile plain tubes under aseptic conditions at weeks 2, 4, 6 and 8 of the experiment. The blood samples were allowed to clot and centrifuged at 3000 rpm for 5 minutes. Analysis was carried out immediately after sample collection. The serum was used for the analysis of total cholesterol, triglycerides, and HDL-cholesterol levels. Total cholesterol was measured using established enzymatic methods (Ferreira *et al*., 2015) with the Biosystem cholesterol kit (Biosystem, Spain). HDLC was estimated by the HDL-C precipitant method (Ferreira *et al*., 2015). Triglyceride was assessed enzymatically (Ferreira *et al*., 2015). LDL-C was calculated using the Friedewald formula (Friedewald *et al.,* 1972).

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA). The differences were separated using Duncan post hoc. The results were presented as mean ± standard deviation of the mean. The level of significance was determined at p<0.05.

Results

The changes in cholesterol level (Figure 1) showed that ovariectomized rats without treatment (group 2) significantly ($p < 0.05$) had higher total cholesterol values than other groups throughout the treatment period. There was no significant difference ($P > 0.05$) in serum total cholesterol level among the rat group that received soy milk powder, the group that received estradiol and the sham group throughout the experimental period.

The changes in serum low density lipoprotein level (Figure 2) in the ovariectomized rat group without treatment (group 2) were significantly higher than the other three groups throughout the experiment. At weeks 4 and 8, the ovariectomized group treated with soy milk (group 1) was significantly ($p < 0.05$) higher than groups 3 and 4 but lower than group 2. At weeks 6, there was no significant difference ($p > 0.05$) between the ovariectomized group treated with soy milk powder (group 1), the ovariectomized estradiol treated group (groups 3) and sham (group 4).

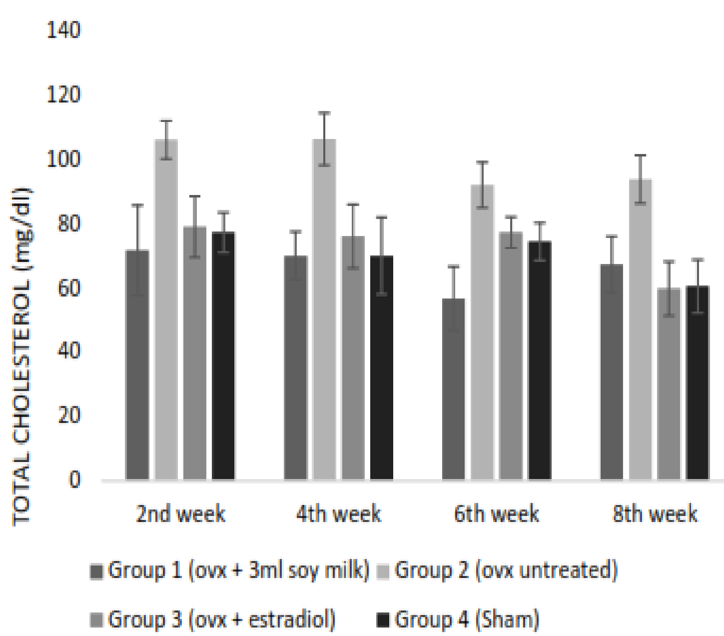

Figure 1: Mean changes in serum total cholesterol (mg/dL) level

The mean serum high-density lipoprotein changes (Figure 3) did not show much difference among the groups. Groups 3 and 4 were higher than the remaining groups 1 and 2 but it was not significant.

The mean serum triglyceride level (Figure 4) showed that the ovariectomized untreated group was significantly higher (p<0.05) than other groups except at week 6 where it was not different from groups 1 and 3.

The soy milk group (group 1) was not significantly different from groups 3 and 4 throughout the experimental period.

Discussion

The reduced estrogen production following menopause results in adverse changes in lipid profile. Estrogens have cardio-protective potential (Shibu *et al***.,** 2017) but this function declines during menopause due to a reduction in circulating estrogen. The standard therapy for the management of these changes is estrogen replacement therapy. Hormone replacement therapy is however associated with negative effects on the recipient (Cagnacci & Venier, 2019). In the present study, the ovariectomized untreated group (group 2) had a significantly higher ($P < 0.05$) total cholesterol level than the sham group (group 4) as well as the ovariectomized groups treated with soy milk powder (group 1) (Figure 1). This finding suggests that ovariectomy alone leads to hypercholesterolemia. It has been reported that ovariectomy causes an increase in the plasma cholesterol level of rats (Camara *et al*., 2014). The groups receiving soy milk powder and estradiol did not show significant differences from the intact group, implying that these treatments may effectively maintain cholesterol levels similar to those in intact rats. This finding is similar to a previous study where soy protein caused a decrease in total cholesterol (Bhattarai *et al.,* 2017). The significant increase in LDL in Group 2 corroborates the impact of ovariectomy on promoting LDL elevation. Both the intact and estradiol-treated groups showed consistently lower LDL levels throughout the experimental period compared to other groups, highlighting the potential benefits of estradiol in mitigating LDL elevation. The soy milk group's LDL levels were slightly higher than those of the estradiol and intact groups at weeks 4 and 8 but still significantly lower than ovariectomized untreated group. This suggests that soy milk may have a role in reducing LDL levels comparable to estradiol. The HDL result in this study did not vary significantly. This result shows that ovariectomy did not adversely affect the HDL of ovariectomized rats and the increase in total serum cholesterol is mainly due to low density lipoprotein. Serum triglycerides from this study showed that the ovariectomized group without treatment was significantly higher than all the other groups. From these changes, the present study, shows that soy milk has an effect on serum lipid concentrations similar to those of estradiol. However, another study in post-menopausal women did not find any effect of soy milk on cholesterol, LDL and HDL (George *et al.,* 2020). The explanation for these differences in findings may be attributed to differences in species and individual metabolism of the isoflavones content of the soy protein. One of the metabolites of diadzein is equol

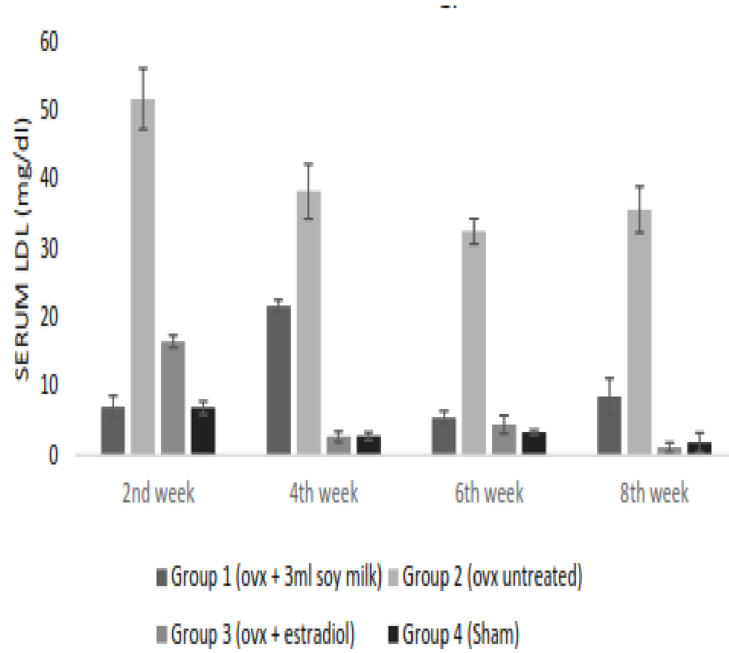

Figure 2: Mean changes in serum low density lipoprotein (mg/dl) level

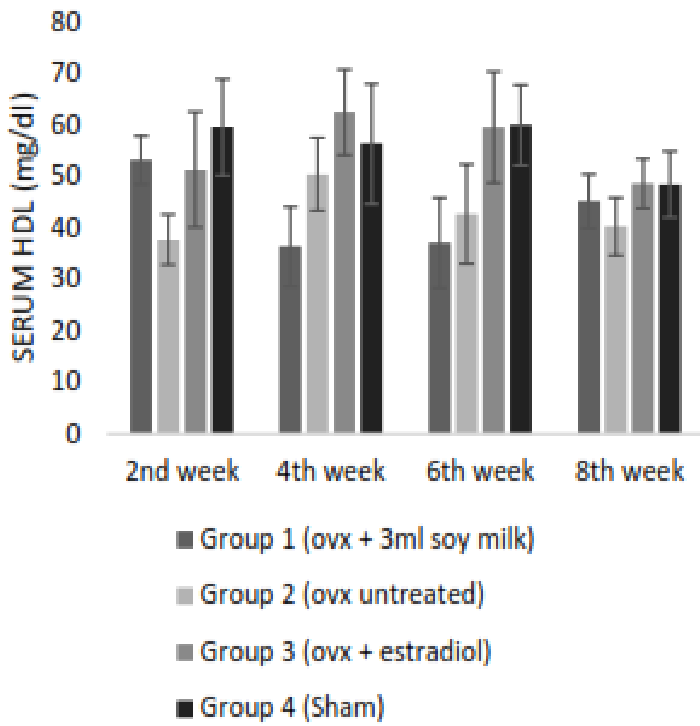

Figure 3: Mean changes in serum high density lipoprotein (mg/dL) level

and some animals have been noted for their ability to convert diadzein to equol (Mayo *et al.,* 2019). Also, some individuals convert diadzein to equol more efficiently than others; for example Asian people have been noted to produce more equol than Western people (Mayo *et al.,* 2019). Two factors have been suggested as the reasons behind the greater effectiveness of equol in comparison to the natural isoflavones present in soy foods. Firstly, equol has a higher affinity for estrogen receptors than its precursor, diadzein. Secondly, equol has more antioxidant capacity than all the isoflavones (Mayo *et al.,* 2019). Production of equol in humans is however dependent on the intestinal microflora. Research has already shown that about one-third of people can produce equol when a high amount of daidzein is consumed (Mayo *et al.,* 2019). A study reported a correlation between equol production and percentage reduction in blood pressure in women (Acharjee *et al.,* 2015). Another factor that may be responsible for the effectiveness of soy milk in these studies may be the timing of intervention which was early (immediately after ovariectomy) in this study. Studies have shown that isoflavone is more effective in preventing bone loss when given in the early stages of menopause than at the late stage (Zheng *et al.,* 2016). Other studies reported that soy protein was more effective in lowering LDL levels in hyperlipidemic individuals than in normolipidemic ones (Sharmin *et al.,* 2017). This means that the effectiveness of soy milk depends on the initial characteristics of the serum lipid of the individual. The result of cholesterol in this study is similar to the findings of George *et al.* (2020), who reported reduced cholesterol in ovariectomized rats fed soy protein as compared to those that received milk. The production of equol as a by-product of isoflavone may have contributed to the efficacy of soy milk in reducing serum cholesterol and LDL in ovariectomized rats.

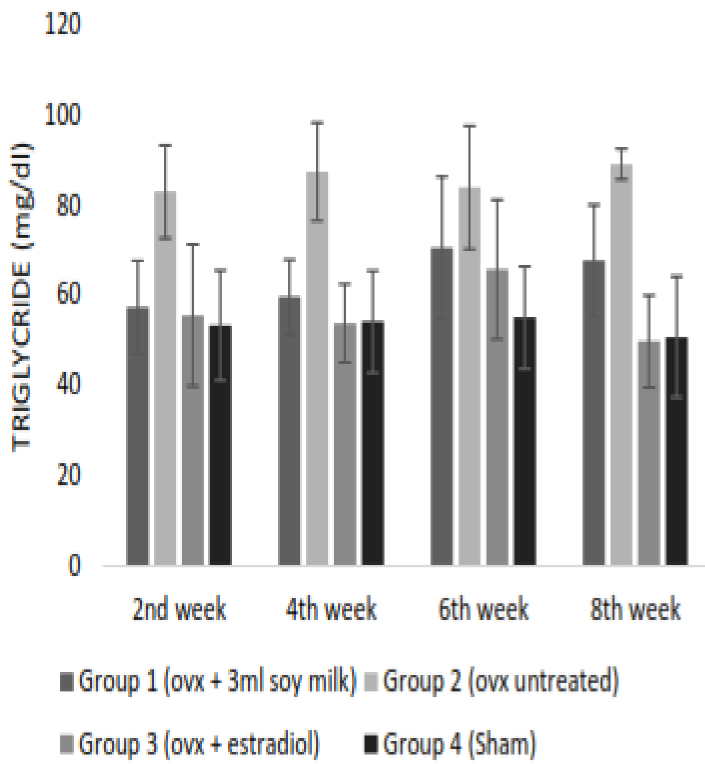

Figure 4: Mean changes in serum triglyceride (mg/dL) level

In conclusion, this study has shown that soy milk containing isoflavone may offer an alternative to hormone replacement therapy using estradiol. Further studies need to be carried out on human patients to ascertain the effect soy milk powder will have on menopausal women. This will be particularly necessary in Nigeria where there is an abundant supply of soybeans and limited access to hormone replacement therapy due to difficulty in accessibility of the hormone and the side effects of the therapy.

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No funding was received.

Conflict of Interest
The authors declare that there is no conflict of interest.

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