Research Article**Effect of *Dalbergia sissoo* on lipid profile and oxidative stress in ovariectomized rats****Ambadas Mane, Sachin Tembhurne*, Swati Kolhe**

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Abstract

Objective: In this study, we tested the effect of hydroalcoholic extract of *Dalbergia sissoo* on oxidative stress, lipid profile in 90 days postsurgical bilateral ovariectomized female rats. **Material and Methods:** 3-month old female Wistar rats were used and distributed in 6 groups: intact groups with 90 days, ovariectomized groups with 90 days, ovariectomized (Ovx) treated hydroalcoholic extract of *Dalbergia sissoo* (200, 350 and 500mg/kg p.o.), ovariectomized treated with standard β Estradiol (0.1mg/kg/s.c). All the treatments were given for further 90 days after post-surgical period (90 days) in ovariectomized female rats. After 90 days of treatments serum estrogen level, level of thiobarbituric acid reactive substances and lipid profile were quantified. **Results:** The treatment of the hydroalcoholic extract of *Dalbergia sissoo* (200, 350 and 500mg/kg) in the Ovx rats shows significant increase in the serum estrogen level, normalization of lipid profile, decreased in the thiobarbituric acid reactive substances. All the results of *Dalbergia sissoo* are comparable with standard β Estradiol. **Conclusion:** Our results suggest that 90 days of treatment with hydroalcoholic extract of *Dalbergia sissoo* is able to increase the estrogen level thereby normalized the lipid profile and oxidative stress in ovariectomized rats.

Keywords: *Dalbergia sissoo*, ovariectomized, estrogen, lipid profile, oxidative stress

Introduction

Ovarian hormone decline after menopause is linked to many pathophysiological reactions. Dyslipidaemia is often seen in postmenopausal women and is characterized by an overall shift toward a more atherogenic lipid profile (Juliana et al., 2011; Rachon et al., 2008). Its incidence increases after menopause due to decreased oestrogen level, since oestrogens are involved in cholesterol metabolism by lowering LDL and increasing HDL concentrations in plasma (Juliana et al., 2011; El-Swefy et al., 2002). It is well known that oestrogens, acting as free radical scavengers, break the free radical chain formation produced from membrane oxidation processes and hence inhibit lipid and protein oxidation (Juliana et al., 2011; Akçay et al., 2000). Estrogens have antioxidant properties and can inhibit lipid peroxidation in vitro (Saeedeh et al., 2013; Arteaga et al., 2003). After menopause, the incidence of cardiovascular disease increases (Saeedeh et al., 2013; Castela et al., 2008). As

antioxidants, isoflavones exert protective effects against cardiovascular disease by decreasing plasma concentrations of thiobarbituric acid reactive substances, which are biomarkers of lipid oxidation, and by increasing the resistance of LDL (Saeedeh et al., 2013; Young et al., 2007; Tsai et al., 1999; Wiseman et al., 2000; Omani et al., 2005). Recently, several animal studies demonstrated that a combined treatment of exercise and an Isoflavone-supplemented diet is more effective against bone loss and fat gain than either treatment alone in estrogen-deficient animals (Wu et al., 2003; Wu et al., 2001; Wu et al., 2004).

Recently, certain plant-derived natural products, mostly phytoestrogens (Isoflavone, lignans, coumestanes, stilbenes, flavonoids) and many more novel estrogen-like compounds in plants has been reported. Although, a number of papers are published on effect of natural products on menopause related osteoporosis, but there is paucity of literature on the accompanying direct effect on other parameters such as lipid profile and oxidative stress in ovariectomized animals with estrogen deficit (Abdullah et al., 2015).

Comprehensive investigation of *Dalbergia sissoo* reported to contain estrogenic flavonoids and some sterols with estrogenic activity. The reported results of phytochemical

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analysis indicated to the presence of flavonoids in *Dalbergia sissoo* (Vikram et al., 2012). Despite the presence of phytoestrogen and flavonoids and antioxidant property of *Dalbergia sissoo*, there are no reports of direct effect on the oxidative stress and lipid profiles due estrogen deficit in ovariectomized female rats. Thus the objective of the present study was to evaluate the effect of hydroalcoholic extract of *Dalbergia sissoo* on lipid profile and oxidative stress in bilateral ovariectomized female rats.

Material and methods

Drugs and chemicals

β -Estradiol benzoate obtained from Analab fine chemicals, 400053. Drug was dissolved and freshly diluted in distilled water to be subcutaneously given.

Preparation of Plant extract

A weighed quantity (50g) of the air dried powdered leaves of *Dalbergia sissoo* was taken and extracted with ethanol (90%) in a Soxhlet extractor. The hydroalcoholic extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50 °C to get a solid residue (Mojahid et al., 2012). Different concentration (200mg/kg, 350mg/kg and 500 mg/kg p.o.) of hydroalcoholic extract of leaves of *Dalbergia sissoo* was according to body weight of animals (Mojahid et al., 2012; Thonda et al., 2013).

Surgical Procedure of bilateral ovariectomy

The acclimatized rats were either sham operated or ovariectomized using the dorsal midline skin incision. The rats underwent surgical procedure after being anaesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg), Rat was put on its ventral surface and ovariectomy was preceded by a single 2 cm long longitudinal skin incision on the dorsal midline (the hump) and the base of tail. After deep incision the bilateral ovaries were found, surrounded by a variable amount of fat. Ligation of blood vessels was necessary. Both ovaries were identified and then silk thread was tightly tied around the oviduct, including the ovarian blood vessels. The oviduct was cut and the ovary was removed, taking good care in leaving the knot intact. The uterine horn was returned in to the abdominal cavity. The muscle incision was required suturing with 0 size chromic absorbable catguts. The skin was sutured with non-absorbable silk thread. The rats of the sham group underwent sham surgery. Sham surgeries received the same incision as the ovariectomized animals and were sutured in the same way but the ovaries were palpated instead of removed. Broad spectrum antibiotic Neosporin antibiotic power was used topically after surgery once a day for 15 consecutive days (Waynforth et al., 1995).

Animal grouping and treatment protocol

After the 3 month, Ovariectomized rats were categorise into six

groups. Group 1- Sham control (without ovariectomized), Group 2- Ovariectomized rats (Ovx), Group 3- Ovx treated with Estradiol (0.1mg/kg/s.c), , Group 4, 5 and 6 - Ovx treated with hydroalcoholic extract of *Dalbergia sissoo* (200, 350 and 500 mg/kg/p.o). All treatments i.e. standard Estradiol (0.1mg/kg/s.c), *Dalbergia sissoo* (200, 350, 500 mg/kg/p.o), respectively were given once for three months.

Estimation of Serum Estradiol Level

Serum Estradiol level measured based on Chemiluminescent Micro particle Immunoassay technique (Yang et al., 2004; Wu et al., 2002).

Determination of Oxidative stress

Oxidative stress in the body measured in terms of lipid peroxidation of unsaturated fatty acids which is commonly used as an index of increased oxidative stress. In the present study elevated level of lipid peroxidation of reactive oxygen species and it measured in the term of TBARS.

After sacrificing, the animals blood were collected from respective groups and centrifuged serum was collected as a source of polyunsaturated fatty acid for determination of extent of TBARS as per previously reported method (Hiroshi et al., 1979; JO et al., 1998; Devasagayam et al., 2003).

Measurement of plasma lipid values

Blood samples were collected from respective group of animals from retro orbital plexus by using anaesthesia, using heparin as the anticoagulant, and plasma was separated by centrifugation (5000 rpm) for 15 min. HDL, LDL and triglycerides were determined by enzymatic colorimetric methods using commercial kits (Biolab diagnostics, Mumbai). Plasma lipids levels were expressed as mg/dl.

Statistical Analysis

Differences in means among the groups in each experimental period were compared by one-way analysis of variance (ANOVA). When significance was indicated, a Tukey's multiple Comparison post hoc analysis was used (Graph Pad Prism Version 5.03).

Results

Serum Estrogen level

The result of serum estrogen level is decreased in ($P<0.0001$) in Ovx group as compared to sham control group. While the treatment with HEDS (200, 350 and 500 mg/kg) significantly ($P<0.0001$) increased in serum estrogen levels as compared to Ovx group. The results of the present study are comparable with standard Estradiol (Figure 1).

Figure 1. Effect of HEDS on serum estrogen level. The values are expressed as Mean $\pm$ SEM (n=6), ###P<0.0001, compared with sham control group; ***P<0.0001; Verses Ovx group (One-way ANOVA followed by Tukey's multiple Comparison test)

Lipid Peroxidation (TBARS estimation)

The result of TBARS is increased in (P<0.0001) in ovariectomized rats as compared to sham control group. While the treatment with HEDS (200, 350 and 500 mg/kg) significantly (P<0.001) decreased in TBARS level dose dependently as compared to ovariectomized group. The results of the present study are comparable with standard β Estradiol (Figure 2).

Figure 2. Effect of HEDS on TBARS level. The values are expressed as Mean $\pm$ SEM (n=6), ###P<0.0001, Verses Control; **P<0.001, ***P<0.0001; Verses Ovx group (One-way ANOVA followed by Tukey's multiple Comparison test)

HDL level

The result of HDL level is decreased in (P<0.0001) in ovariectomized group as compared to sham control group. While the treatment with HEDS (200, 350 and 500 mg/kg) significantly (P<0.0001) increased in HDL dose dependently as compared to ovariectomized group. The results of the present study are comparable with standard β Estradiol (Figure 3).

Figure 3. Effect of HEDS on HDL level. The values are expressed as Mean $\pm$ SEM (n=6), ###P<0.0001, verses sham control; ***P<0.0001, Verses Ovx group (One-way ANOVA followed by Tukey's multiple Comparison test)

LDL levels

The result of LDL level is increased in (P<0.0001) in ovariectomized group as compared to sham control group. While the treatment with HEDS (200, 350 and 500 mg/kg) significantly (P<0.0001) decreased dose dependently in LDL as compared to ovariectomized group. The results of

Figure 4. Effect of HEDS on LDL level. The values are expressed as Mean $\pm$ SEM (n=6), ###P<0.0001, verses sham control; ***P<0.0001, Verses Ovx group (One-way ANOVA followed by Tukey's multiple Comparison test)

the present study are comparable with standard β Estradiol (Figure 4).

Triglycerides level

The result of triglycerides level is increased in ($P < 0.0001$) in ovariectomized group as compared to sham control group. While the treatment with HEDS (200, 350 and 500 mg/kg) significantly ($P < 0.05$ & $P < 0.0001$) decreased in triglyceride level in dose dependent manner as compared to ovariectomized group. The results of the present study are comparable with standard β Estradiol (Figure 5).

Figure 5. Effect of HEDS on Triglyceride level. The values are expressed as Mean $\pm$ SEM ($n=6$), ### $P < 0.0001$, versus sham control; * $P < 0.05$, *** $P < 0.0001$, versus Ovx group (One-way ANOVA followed by Tukey's multiple Comparison test)

Discussion

Menopause is a natural process. After menopause the morbidity and mortality from cardiovascular disease are increased. Lack of oestrogen protection, aging effect, increased body weight, android pattern of body fat distribution seems to be the major cause. In post-menopausal women, there is an increased tendency for body fat deposition in the abdominal region that leads to low HDL and increased LDL. The ovarian hormone deficiency also increases the generation of reactive oxygen species (ROS), which could result in cell damage or death. Many therapies have targeted this hormonal decline in estrogen and have also expanded to include lifestyle modifications. Additionally, foods rich in antioxidants have been shown to be of great benefit in women experiencing menopausal symptoms because they help to eliminate oxidative stress within the body (Joyabrata et al., 2013; Juliana et al., 2011).

As of today, there are several prescription products available for the treatment of postmenopause complications including

dyslipidaemia, obesity, depression, diabetes, osteoporosis etc. most of these are estrogenic agents and combination products. Nevertheless, in view of the lack of effect and/or toxicity of these products, majority of the postmenopausal women are now fascinated by highly publicized natural products. These products are more effective and free from any adverse effects. Recently, certain plant-derived natural products, mostly phytoestrogens (Isoflavone, lignans, coumestanes, stilbenes, flavonoids) and many more novel estrogen-like compounds in plants have been immensely used to prevent menopause-related depletion in bone mineral density (BMD). Although, there is paucity of literature on the lipid profile alteration and oxidative stress in menopause and its treatment.

Comprehensive investigation of *Dalbergia sissoo* reported to contain estrogenic flavonoids and some sterols with estrogenic activity. The reported results of phytochemical analysis indicated to the presence of flavonoids in *Dalbergia sissoo* (Vikram et al., 2012). Owing to the estrogenic nature and the content of flavonoids in plants, the present study was undertaken to evaluate the effects of *Dalbergia sissoo* on Ovariectomized induced dyslipidaemia and oxidative stress.

In women, menopause is associated with decreased HDL. In light of this the present study investigated the level of lipid profile and oxidative stress in terms of lipid peroxidation in ovariectomized rats. In animals, similar results seen that is lower HDL after ovariectomy. While in the treatment group ($P < 0.0001$) significant increasing results have been observed. The HDL levels in the treatment group is comparable with the β Estradiol group of animals.

In women, menopause is associated with increased concentration of LDL. In animals, similar results such as higher LDL after ovariectomy. While in treatment group ($P < 0.0001$) significant decreasing results have been observed. The LDL levels in the treatment group is comparable with the β Estradiol group of animals.

In women, menopause is associated with increased concentration of TG. In animals, similar results such as higher TG after ovariectomy. While in treatment group ($P < 0.0001$) decreasing significant results have been observed. The TG level in the treatment group is comparable with the β Estradiol group of animals.

In healthy, premenopausal women there is usually an appropriate balance between free radical species and antioxidant mechanisms. As such, the level of oxidative stress in these women is not sufficient enough to affect the ovaries until the onset of menopause. It has been stated that menopause creates a pro-oxidant state in the body due to the

decline of the natural antioxidant, estrogen. Consequently, the question often arises if oxidative stress can lead to menopause. The majority of studies have shown that oxidative stress alone in premenopausal women cannot induce menopause, but rather can lead to a variety of pathologies. Specifically, studies have reported that oxygen radicals have an important physiologic role within the ovary (Sejal et al., 2013).

In the present study oxidative stress has been measured in terms of lipid peroxidation by estimating the level of thiobarbituric acid reactive substances (TBARS). The results of the study indicated to increase in the level TBARS in the Ovx group than the sham control group. While in the treatment group ($P < 0.0001$) significant decreasing results observed. The TBARS levels in the treatment is comparable with the β Estradiol group of animals.

Conclusion

From these finding it conclude that hydroalcoholic extract of *Dalbergia sissoo* leaves has estrogenic activity thereby improving the status such as estrogen in ovariectomized rats. Dyslipidaemia and increased in the oxidative stress in ovariectomized rats due to estrogen deficiency while treatment of *Dalbergia sissoo* indicates to show protective action. The effects of *Dalbergia sissoo* on normalization of lipid profile and oxidative stress due to presence of estrogenic activity and presence of flavonoid so it would be helpful in menopausal associated conditions.

Conflicts of interest: Not declared.

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