Effects of *Asparagus racemosus* Root Extracts on Serum Lipid Profiles, Lipid Peroxidation and Superoxide Dismutase in Ovariectomized Rat

Ladachart Taepongsorat¹*, Methin Phadungkit²

**ABSTRACT**

*Background:* Once rats have been ovariectomized they have a high risk of cardiovascular disease due to changes in the blood cholesterol and lipid profile. **Objective:** To investigate the effects of *Asparagus racemosus* (AR) root extract on the serum lipid profiles, lipid peroxidation and antioxidant levels in ovariectomized rats. **Methods:** Twenty-five, two-month-old female Wistar rats were randomly divided into five groups: SH, OVX, OEE, OAAR and OEAR. The daily doses of 500 mg/KgBW of the AR root extract for five weeks. The levels of serum TG, TC, HDL, LDL, the liver, kidney and uterine tissue lipid peroxidation and SOD levels were determined. **Results:** Serum TC and LDL showed no significant differences in any groups. Serum TG of the OAA and OEAR groups were not significantly different. The serum HDL of the OAA and OEAR groups were significantly lower than the OEE group. The liver MDA levels of the OAA and OEAR groups were significantly decreased compared with the OVX and OEE groups while the SOD level of the OAA group was significantly increased. The MDA levels in the kidney and uterine of the treated group showed no significant difference. The SOD levels in the kidney of the treated group were not different but the SOD levels in uterine were significantly decreased. **Conclusion:** It can be believed that the lipid profiles were maybe regulated via estrogen. The AR extract has low effects on the lipid profiles at this dose and duration of treatment. The capacity of the extracts to decrease the MDA level and increase the SOD level in this study clearly reflected the antioxidant efficiency of these substances.

**Key words:** *Asparagus racemosus*, Lipid profiles, Malondialdehyde, Superoxide dismutase, Ovariectomized rat

**INTRODUCTION**

Previous work has indicated that there is an increased risk of coronary heart disease under the menopause as a result of differences in the clotting and fibrinolytic factors, lipid profile and vessel function.¹ For over 30 years blood metabolism has been improved by estrogen replacement. The capacity of the extracts to decrease the MDA level and increase the SOD level in this study clearly reflected the antioxidant efficiency of these substances.

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AR has recently been shown to contain 10 steroidal saponins\textsuperscript{13} and racemofuran.\textsuperscript{14} Rat liver mitochondria were protected by aqueous extracts due to the prevention of radiation-induced loss of protein thiol while SOD was inactivated\textsuperscript{15} along with oxidative stress and hepatotoxicity amelioration.\textsuperscript{16}

The objective of this research was to investigate the effects of Asparagus racemosus root extract (AR) on serum lipid profiles, liver function, renal function, lipid peroxidation and superoxide dismutase (SOD) in ovariectomized rats.

**MATERIALS AND METHODS**

**Plant Material**

The plant roots of AR were collected from Khonkaen province, Thailand and identified by the authors. The voucher specimens were deposited at the Pharmaceutical Chemistry and Natural Product Research Unit, Faculty of Pharmacy, Mahasarakham University, Thailand (MSU. PH-LIL-AR1). The roots were washed thoroughly with tap water and dried at 37°C in an incubator, and then cut into small pieces that were powdered finely before being used for the extractions. The extractions were performed using soxhlet apparatus and concentrated to dryness under a reduce pressure and a controlled temperature using evaporator and a freeze-dryer, respectively. For the experiments, 500 mg/kgBW of the dried extracts were used.

**Experimental Animals and Design**

Female Wistar rats at seven weeks old, weighing approximately 150-180g from the National Laboratory Animal Center, Mahidol University, Salaya Campus, Thailand were used for the experiment. Four rats were housed in one well ventilated standard rat cage under standard conditions (26±2°C, 12 h light-dark cycle) with ad libitum access to drinking water and fed standard rat chow (CP mia feed No.082). The care and procedures adopted for the present investigation were in accordance with the approval of the Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU), Thailand (Approval number 0011/2016).

After a seven day adaptation period, the rats were operated on and randomized into five groups, with five rats in each group, as follows: Group 1; sham group (SH): the same surgical procedure was performed in the sham-operate rats except that the ovaries were not removed. The sham rats received the vehicle without any substance. Groups 2-5; the surgical procedure was performed and the ovaries were removed. Group 2: control group (OVX); OVX rats received the vehicle without any substance. Groups 3-4: treatment groups; OVX rats received 500 mg/kgBW of aqueous extract (OAAR) or ethanolic extract of the AR roots (OEAR), respectively. Group 5; OVX rats received 17α-ethynylestradiol 0.1 mg/kgBW for a positive control (OEE). Each rat received their substances by feeding needle, daily for five weeks. All treatments were initiated precisely 10 days after surgery. Their body weights were recorded every day during the experimental period. At the end of the experiment, the rats were sacrificed under anesthesia and blood was collected immediately by cardiac puncture; serums were separated by centrifugation. The liver, kidney and uterus were excised, weighed and kept at -80°C until analyzed.

**Biochemical parameters analysis**

Biochemical parameters, such as blood glucose (BG), liver function test: serum aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (ALT), renal function test: blood urea nitrogen (BUN), creatinine (Cr) and lipid profiles: triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL) were measured in a Clinical Automatic Chemistry Analyzer (Tecom 6060 L) at the M-LAB, Mahasarakham laboratory, Thailand using standard laboratory techniques.

**Lipid peroxidation and SOD antioxidant enzyme activity**

The liver, kidney and uterus tissues were homogenized in a potassium chloride buffer with a tissue homogenizer and lipid peroxidation was estimated according to the method of Ohkawa.\textsuperscript{17} Concentrations were determined from a standard curve using Tetramethoxpropane (TMP) and expressed as nmol of malondialdehyde (MDA) formed per 100 mg protein of tissue. The method of Lowry et al.\textsuperscript{18} was used to examine the protein content.

Superoxide dismutase (SOD) activities in liver, kidney and uterine homogenates were determined using the nitrobluetetrazolium reduction method with Sigma-Aldrich determination kit (product number 19160).

**Statistical analysis**

All data were presented as a mean ± SEM. A one-way ANOVA followed by a Student’s t-test were used to determine the statistical significance. P-values < 0.05 were considered as statistically significant.

**RESULTS**

**Vaginal cellular differentiation**

Daily vaginal smear cytology was used to identify the estrous cycle phases of the rats, as regular cycles are required. The vaginal smear was examined from all rats every morning for 35 days (five weeks). Sham rats had a normal estrous cycle. Until the experiment was completed, the ovariectomized rats showed the diestrous phase (leukocyte cells). In the positive control rats, vaginal cells showed maturation in response to 17α-ethynylestradiol at a dose of 0.1 mg/kgBW on the third day during the 35 day exposure period. Both the aqueous and ethanolic extracts of the AR roots induced significant cornification cells when compared to the OVX group on the fifth day of the exposure period.

**Table 1:** Body weight, uterine weight, relative uterine weight, liver weight, relative liver weight, kidney weight and relative kidney weight (mean ± SEM).

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Uterine weight (g)</th>
<th>Relative uterine weight (x100)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight (x100)</th>
<th>Kidney weight (g)</th>
<th>Relative kidney weight (x100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>266±11</td>
<td>0.35±0.02</td>
<td>0.13±0.00</td>
<td>7.23±0.29</td>
<td>2.73±0.14</td>
<td>1.50±0.09</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td>OVX</td>
<td>288±9*</td>
<td>0.17±0.01*</td>
<td>0.06±0.00</td>
<td>7.50±0.35</td>
<td>2.60±0.07</td>
<td>1.56±0.02</td>
<td>0.54±0.01</td>
</tr>
<tr>
<td>OAAR</td>
<td>273±6*</td>
<td>0.25±0.03*</td>
<td>0.09±0.00*</td>
<td>6.41±0.09*</td>
<td>2.36±0.05*</td>
<td>1.36±0.03*</td>
<td>0.50±0.00*</td>
</tr>
<tr>
<td>OEAR</td>
<td>276±8*</td>
<td>0.24±0.02*</td>
<td>0.09±0.01*</td>
<td>6.86±0.24</td>
<td>2.50±0.14</td>
<td>1.47±0.09</td>
<td>0.53±0.03</td>
</tr>
<tr>
<td>OEE</td>
<td>224±6*</td>
<td>0.43±0.04*</td>
<td>0.19±0.01*</td>
<td>7.69±0.43</td>
<td>3.42±0.11*</td>
<td>1.34±0.07*</td>
<td>0.59±0.02</td>
</tr>
</tbody>
</table>

*, †, ‡ show significant differences (p<0.05) compared with SH, OVX and OEE groups, respectively.
Table 2: Serum biochemical levels (mean ± SEM).

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>BUN (mg/dl)</th>
<th>Cr (mg/dl)</th>
<th>BG (mg/dl)</th>
</tr>
</thead>
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<tr>
<td>SH</td>
<td>153.7±20.5</td>
<td>41.5±8.21</td>
<td>20±1.75</td>
<td>0.53±0.04</td>
<td>103±11.28</td>
</tr>
<tr>
<td>OVX</td>
<td>147.40±11.41</td>
<td>34.8±3.25</td>
<td>22±0.59</td>
<td>0.48±0.04</td>
<td>107±4.58</td>
</tr>
<tr>
<td>OAAR</td>
<td>148.60±15.51</td>
<td>33.6±5.35</td>
<td>20±0.39</td>
<td>0.55±0.07</td>
<td>111±2.01</td>
</tr>
<tr>
<td>OEAR</td>
<td>116.80±14.94</td>
<td>30.2±5.16</td>
<td>19±1.59</td>
<td>0.47±0.04</td>
<td>109±3.79</td>
</tr>
<tr>
<td>OEE</td>
<td>123.80±10.35</td>
<td>37.0±3.69</td>
<td>23±2.68</td>
<td>0.45±0.03</td>
<td>111±7.44</td>
</tr>
</tbody>
</table>

Table 3: Lipid profile levels in serum (mean ± SEM).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>53.75±8.38</td>
<td>119±3.74</td>
<td>35.2±3.04</td>
<td>54.73±7.21</td>
</tr>
<tr>
<td>OVX</td>
<td>67.4±6.46</td>
<td>110.8±6.87</td>
<td>36.18±2.29</td>
<td>67.70±6.12</td>
</tr>
<tr>
<td>OAAR</td>
<td>65.0±5.52</td>
<td>111.6±3.55</td>
<td>36.74±1.94</td>
<td>61.42±4.63</td>
</tr>
<tr>
<td>OEAR</td>
<td>71.2±7.13</td>
<td>106.8±3.06</td>
<td>39.96±3.76</td>
<td>64.92±5.12</td>
</tr>
<tr>
<td>OEE</td>
<td>69.0±6.36</td>
<td>114.8±6.63</td>
<td>51.98±3.66*</td>
<td>53.82±7.43</td>
</tr>
</tbody>
</table>

* # p show significant differences (p<0.05) compared with SH, OVX and OEE groups, respectively.

Body Weight and Organs Weight

The body and organs weight are in Table 1. The OVX group had rapid weight gain in the week following ovariectomy, which remained higher than every other group for five weeks. At the end of the experiments, the OVX group tended to increase in final body weight when compared to the SH and both treated groups. Body weight in the OEE group began to significantly decrease in the first week after the start of the treatment when compared with OVX group. The SH and treatment groups showed a gain in body weight but this alteration was less than that in the OVX rats. The treatment with aqueous and ethanolic extracts significantly increased in the body weight when compared to the positive control group.

The changes in the uterine weight and relative uterine weight are shown in Table 1. The OVX group had the significantly lowest uterine weight and relative uterine weight. The uterine weight in the OEE group was significantly increased when compared with the OVX group, whereas the relative uterine weight in the OEE group was also significantly increased when compared with the OVX and SH groups. Whereas uterine weight and relative uterine weight in the OAAR, OEAR groups were significantly decreased when compared with the SH and OEE groups. The uterine weight and relative uterine weight of the treated groups were significantly increased in comparison with the OVX group.

The liver weight and relative liver weight of the OVX group were not significantly different when compared with the SH, OEE and OEAR groups. In the OAAR group, liver weight and relative liver weight were significantly lower than in the SH, OVX and OEE groups. The relative liver weight of the OEE group was increased significantly when compared with the SH and OVX groups. The relative liver weight of the OAAR group was significantly lower than the OEE group. The relative liver weight of the OEAR was decreased significantly when compared with the OEE group.

The kidney weight and relative kidney weight of the OVX group had no significant differences when compared with the SH, OEE and OEAR groups. The kidney weight of the OEE and OAAR groups decreased significantly when compared with the OVX group. The relative kidney weight of the OAAR group decreased when compared with the SH, OVX and OEE groups.

Effects of extracts on serum AST, ALT, BG, BUN, Cr

The results of the blood biochemical tests in rats are shown in Table 2. The serum AST, ALT, BUN, Cr and BG did not change with the treatment.

These results showed that the extract at a daily dose of 500 mg/kgBW in ovariectomized rats for five weeks had no effects on liver or kidney function and serum blood glucose.

Effects of extracts on serum lipid profiles

The effects of the AR root extracts on the serum lipid profiles are summarized in Table 3. Compared with the SH group, serum TC and LDL tended to increase in all groups. However, serum HDL and LDL showed no significant differences in any groups.

In the OEAR group, the serum TG level was significantly lower than in the SH group. The serum TG of the OAAR and OEAR groups were not significantly different when compared with the OVX group.

The serum HDL level in the OEE group was significantly higher than in the SH and OVX groups. The serum HDL of the OAAR and OEAR groups was significantly lower than the OEE group.

Effects of extracts on MDA and SOD

The levels of MDA and SOD in the tissues are shown in Table 4. In liver, the MDA level was significantly increased, whereas the SOD level was significantly decreased in the OVX and OEE groups when compared with the SH group. The MDA levels in the liver of the OAAR and OEAR groups were significantly decreased when compared with the OVX and OEE groups. The SOD level in the liver of the OAAR group was significantly increased when compared with the OVX and OEE groups. The SOD level in the liver of the OEAR group was significantly decreased when compared with the SH group.

The liver weight and relative liver weight of the OVX group were not significantly different when compared with the SH, OEE and OEAR groups. In the OAAR group, liver weight and relative liver weight were significantly lower than in the SH, OVX and OEE groups. The relative liver weight of the OEE group was increased significantly when compared with the SH and OVX groups. The relative liver weight of the OAAR group was significantly lower than the OEE group. The relative liver weight of the OEAR was decreased significantly when compared with the OEE group.

The kidney weight and relative kidney weight of the OVX group had no significant differences when compared with the SH, OEE and OEAR groups. The kidney weight of the OEE and OAAR groups decreased significantly when compared with the OVX group. The relative kidney weight of the OAAR group decreased when compared with the SH, OVX and OEE groups.

Effects of extracts on serum lipid profiles

The effects of the AR root extracts on the serum lipid profiles are summarized in Table 3. Compared with the SH group, serum TC and LDL tended to increase in all groups. However, serum HDL and LDL showed no significant differences in any groups.

In the OEAR group, the serum TG level was significantly lower than in the SH group. The serum TG of the OAAR and OEAR groups were not significantly different when compared with the OVX group.

The serum HDL level in the OEE group was significantly higher than in the SH and OVX groups. The serum HDL of the OAAR and OEAR groups was significantly lower than the OEE group.

Effects of extracts on MDA and SOD

The levels of MDA and SOD in the tissues are shown in Table 4. In liver, the MDA level was significantly increased, whereas the SOD level was significantly decreased in the OVX and OEE groups when compared with the SH group. The MDA levels in the liver of the OAAR and OEAR groups were significantly decreased when compared with the OVX and OEE groups. The SOD level in the liver of the OAAR group was significantly increased when compared with the OVX and OEE groups. The SOD level in the liver of the OEAR group was significantly decreased when compared with the SH group.
The MDA and SOD levels in the kidney of the OVX group showed no significant differences. The MDA levels in the kidney tissue of the OEE group were significantly decreased when compared with the SH group, while the SOD level was significantly increased when compared with the SH group. The MDA levels in the kidney tissue of the OAAR group were significantly decreased when compared with the SH group, whereas the SOD showed no significant difference. The SOD level in the kidney tissue was significantly decreased in the OEAR when compared with the OEE group.

The MDA levels in the uterine of the O VX, OAAR and OEE groups showed no significant differences. In the OEAR group, the MDA and SOD levels in the uterine tissue were significantly increased when compared with the OEE group. The SOD level in the uterine tissue were significantly increased in the OVX group and tended to increase in the OAAR and OEAR groups. The SOD level in the uterine tissue was significantly decreased in the OEE group.

DISCUSSION

Cornification of the epithelial cells was induced via synthetic estrogens and phytoestrogens and their estrogenic activity. After ovariectomy, all the rats had leucocyte populations affirming the complete removal of the ovaries. Vaginal smears of O VX rats did not show any cornification proving the absence of endogenous estrogens. In this study, cornification cells in positive control, OEE group responded to 17α-ethynylestradiol at a daily dose of 0.1 mg/kgBW on the third day, 17β-ethynylestradiol at a daily dose of 50 µg/kgBW and estradiol valerate at a dose of 1 mg/kgBW on the fifth day of the exposure period. Both aqueous and ethanolic extracts of the root of AR induced significant cornification of cells on the fifth day of the exposure period. In this study, the cornification of the vaginal smear in the OVX rats treated with the AR extract can be attributed to its estrogenic potential.

Estrogen was found to have an important regulatory effect on female adipose deposition and phytoestrogens and their estrogenic activity. After ovariectomy, ovaries shrink and result in a number of deleterious effects. In women, the menopause is associated with increased concentrations of TC, TG, LDL and decreased concentrations of HDL. In vivo, menopause is associated with increased concentrations of TC, TG, LDL and decreased concentrations of HDL. The toxic free radicals can be removed by scavenging enzymes, such as SOD that removes superoxide radical. Oxygen free radicals damage membranes and biological structures, but the damage can be prevented by SOD that removes superoxide radical. The toxic free radicals can be removed by scavenging enzymes, such as SOD and CAT, in vivo. Reduced activities of SOD in the liver have been observed in ovariectomized rats and this results in a number of deleterious effects due to the accumulation of super oxide radicals. Oxidative stress was found to be inhibited by the AR root extracts as assessed by a decrease in liver and kidney MDA levels. Ovariectomized rats treated with 17α-ethynylestradiol were found to have increased triglyceride and HDL levels. Ovariectomized rats treated with AR root extracts were not significantly different in the lipid profile with AR root powder. In this study, male rats administered with the aqueous and ethanolic extracts of the AR roots also had no significant changes in their lipid and antioxidant profiles. Estrogen increased HDL and TG while decreasing LDL and fat deposition in OVX animal models. As for the lipid profile, many studies have reported that the lipid profile undergoes changes during menopause and ovariectomy.

There were no significant changes in the lipid profiles of male rats administered with the AR root powder. In this study, female rats administered with the aqueous and ethanolic extracts of the AR roots also had no significant changes in their lipid and antioxidant profiles. Estrogen increased HDL and TG while decreasing LDL and fat deposition in OVX animal models. As for the lipid profile, many studies have reported that the lipid profile undergoes changes during menopause and ovariectomized. In women, the menopause is associated with increased concentrations of TC, TG, LDL and decreased concentrations of HDL. In the present study, TG and HDL levels were increased in ovariectomized rats treated with 17α-ethynylestradiol, but there were no significant differences in TC and LDL levels. Camara et al. and Antunes et al. indicated that in ovariectomized rats there were no significant changes in the levels of TC and LDL as in our present study. There was a significant difference in the lipid profiles between the OEE and SH groups. From these results it is possible that the lipid profile is regulated due to estrogen. In addition, estrogen therapy is well known to reduce body weight and fat deposition and result in favorable changes in plasma lipid profiles.

Oxidative stress was found to be inhibited by the AR root extracts as assessed by a decrease in liver and kidney MDA levels. Ovariectomized rats treated with 17α-ethynylestradiol were found to have increased triglyceride and HDL levels. Ovariectomized rats treated with AR root extracts were not significantly different in the lipid profile levels compared with the SH and OEE groups. Ovariectomized rats had no significant differences in HDL, LDL and TG but had increased TC. The estrogen group had elevated levels of LDL and TC. There were increases in the HDL levels and decreases in the LDL levels that were greater than those expected just from the postmenopausal period and old age. In this study, AR did not differ in the lipid profiles.
rats exhibited elevated levels of MDA and enzymic antioxidants SOD in uterus. Elevations in the SOD activity may be a compensatory mechanism for chronic overproduction of free radicals and oxidative stress in uterine of ovariectomized rats. The precipitation of cholesterol can be induced in vitro via steroid glycosides, such as digitonin and tomatine, but they also prevent in vivo absorption affecting bile acid, the size and structure of micelles can be affected by several triterpenoid saponins, which causes changes in the absorption of bile acid as well inhibiting the absorption of cholesterol. In addition, saponins can result in reduced plasma LDL levels via the turnover of LDL to hepatic tissue being increased, and this becomes bile acid. Saponins have been reported to prevent pancreatic lipase activity by lowering TG, and from this the VLDL levels reduce there could be a direct link to TG levels decreasing. However, this current work does not suggest any significant alterations on the TG or VLDL levels in the studied groups, and the possible mechanism for this is still unknown.

This work found that there were no significant differences between any of the groups for the TC in the serum. Due to saponins interfering with the enterohepatic circulation of bile acids and precipitating cholesterol from micelles they can prevent the intestinal absorption of cholesterol that limits the plasma cholesterol levels.

CONCLUSION

The results presented here support the use of estrogen in regulation of lipid profiles. AR root extracts have low effects on lipid profiles under this dose and duration treatment used here. The capacity of the extracts to decrease the MDA level and increase the SOD levels in this study clearly reflect the antioxidant efficiency of the AR root extracts.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ALT: Serum glutamate pyruvate transaminase; AR: Asparagus racemosus; AST: Serum aspartate aminotransferase; BG: Blood glucose; BUN: Blood urea nitrogen; Cr: Creatinine; HDL: High density lipoprotein; LDL: Low density lipoprotein; MDA: Malondialdehyde; SOD: Superoxide dismutase; TC: Total cholesterol; TG: Triglyceride; SH: Sham-operated rats group; OVX: Ovariectomized rats group; OAAR: Ovariectomized rats + aqueous extract group; OEAR: Ovariectomized rats + ethanolic extract group; OEE: Ovariectomized rats + 17α-ethynlestradiol group.

REFERENCES