



The Evaluation of Retinol, α -Tocopherol, Cholecalciferol and Reproductive Hormones Levels After Administrated *Allium Schoenoprasum* L. Ethanol Extract and Acrylamide in the Female Rats

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ABSTRACT

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This study was carried out to determine the levels of retinol, α -tocopherol, cholecalciferol and reproductive hormones (follicle stimulating hormone - FSH, luteinizing hormone - LH, progesterone, estradiol) in the female rats administrated *Allium schoenoprasum* L. ethanol extract (ASLEE) and acrylamide. The study was performed on thirty-two Wistar albino female rats (200-220 grams). The rats were divided into 4 groups with an equal number. Serum LH values were higher in the ASLEE group compared to the other groups. Control group: No treatment was performed. Acrylamide group: Acrylamide was administrated by gastric gavage at a dose of 25 mg / kg daily. ASLEE group: ASLEE was administrated by gastric gavage at a dose of 200 mg / kg daily. Acrylamide + ASLEE group: Acrylamide was administrated by gastric gavage at a dose of 25 mg / kg daily. Then ASLEE was administered by gastric gavage at a dose of 200 mg / kg per day. Serum FSH and LH values were significantly lower in the acrylamide group compared to the other groups. Serum LH values in the acrylamide + ASLEE group were significantly restored compared to the acrylamide group. Serum estradiol values were partially lower in the acrylamide group compared to other groups, but there was no significant difference between the groups. Serum progesterone values in the acrylamide group were significantly lower than the control group. Serum progesterone values were higher in the acrylamide + ASLEE group compared to the acrylamide group. As a result, the levels of retinol, α -tocopherol, cholecalciferol and reproductive hormones in ASLEE were determined in this study. In addition, when ASLEE was applied with acrylamide, the rate of change in the relevant parameters was determined.

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Introduction

Acrylamide is a colourless, odourless substance found in solid state in nature (Friedman et al., 2003). Acrylamide, which penetrates into the blood through the digestive tract or via the skin, is known to be carcinogenic or neurotoxic. Acrylamide is formed by exposure of carbohydrate-rich foods to temperatures exceeding 120°C (Rommens et al., 2008). Ready-made foods such as crackers, toast and chips are reported to form acrylamide due to the way they are produced (Viklund et al., 2008). Wei et al. (2014) reported that acrylamide (20 mg / kg and 40 mg / kg) administered

to prepubertal female rats for 30 days caused decreases in body and organ (ovary and uterus) weights and also reduced the number of corpus luteum. In another study, it was reported that male rats receiving oral acrylamide for 8 weeks had decreased body weight and epididymal sperm reservoirs (Wang et al., 2010).

The vast majority of natural sources have been reported to have biological activities (Sevindik et al., 2017; Mushtaq et al., 2020; Mohammed et al., 2020; Sevindik, 2020). *Allium schoenoprasum* L. (Sirmo), a member of the family

Lilyceae, is known as the chives (Parvu et al. 2014). *Allium schoenoprasum* L. has been reported to have antitumoral (Shirshova et al., 2014), antidiabetic, antioxidant effects (Çelik et al., 2008, Zeng et al., 2017). *Allium schoenoprasum* L. (Sirmo) ethanol extract has been reported to exert neuroprotective effects in mice by enhancing antioxidant defense against ischemia reperfusion injury (Singh et al., 2018).

This study was performed to evaluate the reproductive hormones (follicle stimulating hormone - FSH, luteinizing hormone - LH, progesterone, estradiol) and some vitamin (retinol, α -tocopherol, cholecalciferol) levels in female rats administrated *Allium schoenoprasum* L. (Sirmo) ethanol extract and acrylamide.

Materials and Methods

Animals

The study was approved by the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (Approval No: 2020/01). Ethical rules were taken into consideration in all applications. Thirty-two female Wistar albino rats (200-220 grams) were used in the study. The rats were housed under standard laboratory conditions (12 hours light / 12 hours dark). There was no restriction on feed and water.

Chemicals

Acrylamide (Sigma for electrophoresis, $\geq 99\%$, CAS No 76-06-1) were purchased from a pharmacy.

Preparation of Plant Extract

Allium schoenoprasum L. was collected in May and then dried in the shade. 300 g of *A. schoenoprasum* was then ground in an electric mill and pulverized. It was then suspended in 4 liters of 80% ethanol for 3 days and passed through Watman filter paper and evaporated in a rotary evaporator at 50°C with ethanol (Koçak et al., 2019).

Experimental Design

The study lasted 15 days. Rats were divided into 4 groups.

- Control group (n=8): No treatment was performed.
- Acrylamide group (n=8): Acrylamide was given by gastric gavage at a dose of 25 mg / kg daily (Altinoz and Turkoz, 2014).
- A *Allium schoenoprasum* L. (Sirmo) group (n=8): *Allium schoenoprasum* L. (Sirmo) ethanol extract was given by gastric gavage at a dose of 200 mg / kg daily (Aamir et al., 2016).
- Acrylamide + *Allium schoenoprasum* L. (Sirmo) group (n=8): Acrylamide was given by gastric gavage at a dose of 25 mg / kg daily. Then *Allium schoenoprasum* L. (Sirmo) ethanol extract was administered by gastric gavage at a dose of 200 mg / kg per day.

At the end of the study, rats were sacrificed by high blood collection. Blood samples were taken into biochemistry tubes.

Hormonal Analysis

Serum estradiol, FSH, LH and progesterone measurements were performed on the Abbott Architect I16200TM using chemiluminescence microparticle immunological method using the appropriate calibrator, control and kit. Serum estradiol levels were expressed as pg / mL, FSH and LH levels were expressed as mIU / mL and progesterone levels were expressed as ng / mL.

Plasma Extraction

For the analysis of retinol, α -tocopherol, cholecalciferol, 200 μL plasma was taken into plastic tubes. 200 μL of ethanol was added and vortexed for 1 mi. Then, it was centrifuged at 2000 RPM for 10 minutes. 800 μL of the resulting hexane phase were taken and dried under nitrogen gas. The residue was dissolved in 100 μL of methanol and injected into the HPLC column (Miller and Yang, 1985; Zaspel and Csallany, 1983).

Liquid Chromatography

First, the instrument was prepared for analysis using retinol, α -tocopherol, cholecalciferol standards. Then 100 μL of the extracts were injected into the liquid chromatography column. Diagnosis of retinol, α -tocopherol, cholecalciferol was made using diode-array detector at 325, 265 and 290 nm wavelengths. Methanol-water (98: 2) was used as the mobile phase at a flow rate of 1.5 mL / min. The C18 column (4.6 mm x 25 cm) was used for the separation of vitamins (Miller and Yang, 1985; Zaspel and Csallany, 1983; Reynolds and Judd, 1984). Analyses were performed with Agilent 1100 series HPLC instrument.

Statistical analysis: SPSS (version 20) was used for analysis. For the differences between the groups, Kruskal-wallis, one of the non-parametric analysis methods, was preferred ($P < 0.05$). OneWay ANOVA was used to determine which group caused the difference. Then, post-hock multiple comparison test (Tukey HSD) was used. Means with a P value of 0.05 or less were considered significant compared to each other.

Results

Serum LH levels were higher in ASLEE group compared to all groups ($P < 0.001$). However, serum LH levels decreased dramatically in the acrylamide group compared to the other groups ($P < 0.001$). Serum LH levels were significantly restored in the acrylamide + ASLEE group compared to the acrylamide group ($P < 0.001$).

When the serum FSH values were examined, the serum FSH values of the acrylamide group were lower than the other groups ($P < 0.020$), but there was no significant difference between the serum FSH values of the other groups ($P > 0.05$). In addition, serum estradiol levels were slightly lower in the acrylamide administrated groups compared to the other groups, but there was no significant difference between the groups ($P > 0.05$).

Table 1. Reproductive hormone values of all groups.

Parameters	LH (mIU/L)	FSH (mIU/L)	Estradiol (pg/mL)	Progesterone (ng/mL)
Control	8.50±0.55	47.17±7.19	29.83±5.74	63.27±2.66
Acrylamide	3.84±0.45*	39.17±4.22 [‡]	26.41±2.23	49.50±2.64*
ASLEE	10.16±0.46*	49.17±1.17	29.69±1.44	62.17±2.10
Acrylamide+ASLEE	7.79±0.32	43.67±4.93	27.17±1.72	54.48±2.64*
P values	0.001	0.020	0.072	0.001

*P: Significant compared to other groups (P=0.001), [‡] P: Significant compared to control and ASLEE groups (P=0.020). LH: luteinizing hormone (mIU/L), FSH: follicle stimulating hormone (mIU/L), estradiol (pg/mL), progesterone (ng/mL).

When the condition was examined in terms of progesterone results, a situation similar to LH values appeared. Serum progesterone levels were significantly lower in the acrylamide administrated groups compared to the control group (P<0.001). Serum progesterone levels were higher in the acrylamide + ASLEE group compared to the acrylamide group and approached the control group values (P<0.001). The results are detailed in Table 1.

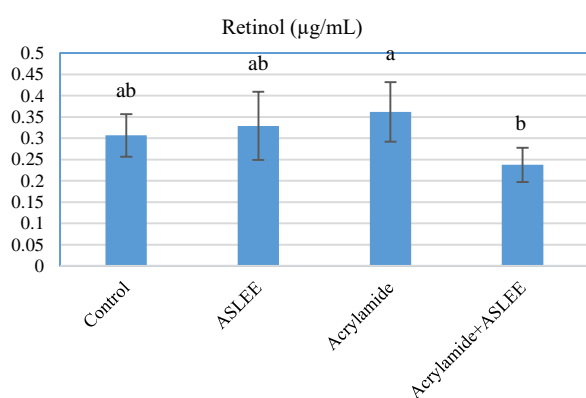


Figure 1. Comparison of retinol values between groups

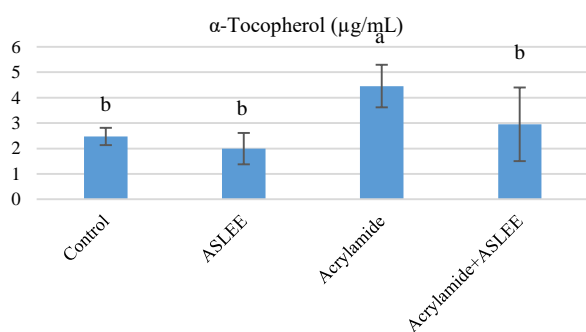


Figure 2. Comparison of α-tocopherol values between groups

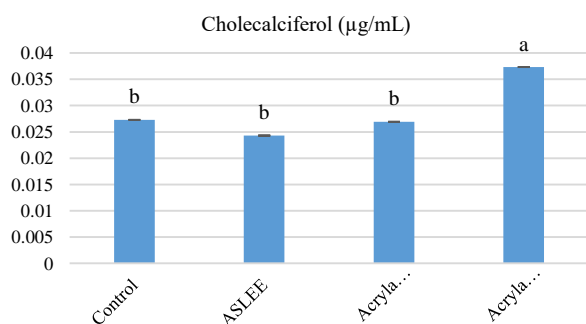


Figure 3. Comparison of cholecalciferol values between groups

Retinol and α-tocopherol levels were determined as highest in the acrylamide group. There was no significant difference between the control and ASLEE groups in terms of retinol and α-tocopherol levels. Cholecalciferol level was significantly higher in acrylamide + ASLEE group compared to the other three groups. However, there was no significant difference in terms of cholecalciferol levels between acrylamide, control and ASLEE groups. Detailed results regarding retinol, α-tocopherol and cholecalciferol are presented in Figure 1, Figure 2 and Figure 3. Different letters (a, b) indicate significant inter-group differences (P<0.05).

Discussion

It is known that plant extracts are used in many studies and they have therapeutic power due to some features. In our study, acrylamide, which is revealed after carbohydrate-rich foods were cooked at high temperatures, is used. Also, ASLEE also known as Sirmo, is the basis of our study.

Although the serum estradiol values in the current study were partially lower in the acrylamide groups compared to other groups, there was no significant difference between the groups (P>0.05). This result is compatible with previous study results (Hamdy et al., 2017, Wei et al., 2014). Wei et al. (2014) also reported that when they administered acrylamide orally for 30 days, it significantly reduced the number of corpora lutea, body weights and organ weights. Our conclusion regarding estradiol differ from the result of Mannaa et al. (2006). Mannaa et al. (2006) reported that in the acrylamide group, the level of estradiol significantly decreased compared to the control group. This difference between the two studies can be attributed to the differences in study times and acrylamide doses.

In our study, serum progesterone values were found to be significantly lower in acrylamide-administrated groups than in the control group (P<0.001). In our study, it was determined that serum progesterone value was higher in Acrylamide + ASLEE group than acrylamide group. Also, Wei et al. (2014) reported that serum progesterone concentrations decreased significantly with increasing acrylamide doses in their studies (P<0.05). Mannaa et al. (2006) reported that the progesterone value in the acrylamide group was significantly lower than the control group. However, Hamdy et al. (2017) reported that, unlike our study, the value of progesterone did not differ significantly between groups.

LH and FSH values were highest in the ASLEE group and lowest in the acrylamide group. Significant increase in serum LH and FSH values in Acrylamide + ASLEE group compared to acrylamide group shows the effect of ASLEE on LH and FSH. After the literature review, it was

determined that LH and FSH parameters were not evaluated in either acrylamide or ASLEE studies. For this reason, the findings we obtained regarding LH and FSH in our study will remain as significant data.

In our study, we applied Acrylamide and ASLEE to rats and evaluated the levels of retinol, α -tocopherol and cholecalciferol. To date, no studies on these parameters have been performed in any living creature administrated Acrylamide and ASLEE. Therefore, we have no chance to compare our data. In the current study, retinol and α -tocopherol levels were found to be highest in the acrylamide group. There was no significant difference between retinol and α -tocopherol levels in the control and ASLEE groups. While it was determined that cholecalciferol level was significantly higher in Acrylamide + ASLEE group than the other three groups, there was no significant difference between these three groups.

Conclusion

As a result, with the current study, retinol, α -tocopherol and cholecalciferol levels, and LH and FSH levels were determined in rats administrated with acrylamide and ASLEE. We think that these results will shed light on future studies in this field and contribute to the literature.

Conflict of interest

There is no conflict of interest in this study.

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