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THE EFFECT OF GINGER EXTRACT ON ANTIOXIDANT, SEXUAL HORMONES AND UTERUS AND OVARY HISTOLOGY PARAMETERS OF FEMALE RATS EXPOSED TO OXIDATIVE STRESS

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ABSTRACT

This study was conducted to determine the effect of ginger extract on antioxidant, sexual hormones and uterus and ovary histology parameters of female rats exposed to oxidative stress. 20 newly weaned female rats were divided into 4 groups. The first group (control) received olive oil at a dose of 0.1 ml through gastric gavage for 30 days. The second group received olive oil at a dose of 0.1 ml through gastric gavage for 30 days as well as 0.2 ml/kg BW (TBH) by an intraperitoneal injection every 2 days. The third group received the dissolved extract of hydro-alcoholic ginger in 0.1 ml olive oil as a gastric gavage in 5 days of a week and for 30 days and the fourth group received 0.2 ml TBH by an intraperitoneal injection every 2 days and for 30 days and they also received hydro-alcoholic extract of ginger in 0.1 ml olive oil by a gastric gavage in 5 days of a week and for 30 days. At the end of the experiment, sexual hormones of FSH, LH, estradiol and progesterone were measured in plasma. The results showed that the interactions between hydro-alcoholic extract of ginger and TBH injection on the studied hormones were not significant. TBH injection to the mice reduced the concentration of LH and estradiol. The concentrations of LH and FSH of the mice that had received the hydro-alcoholic extract of ginger increased. The interactions between hydro-alcoholic extract of ginger and TBH injection were not significant. TBH injection led to ALT increase. The concentration of liver enzymes in mice that received the hydro-alcoholic extract of ginger did not change. The interactions between hydro-alcoholic extract of ginger and TBH injection was significant only in MDA index. TBH injection to mice led to increase MDA index and reduce the percentage of

total antioxidant capacity. The release rate of free radicals in mice which received the hydro-alcoholic extract of ginger showed increase. In the pathology results of uterus and ovary tissues, we can point to a particular apoptosis in the ovary of TBH injected group and the group that received the hydro-alcoholic extract of ginger associated with TBH injection. A significant reduction in endometrial and also deformation of endometrial lining tissue from the short cylindrical and even cubic shape were observed in the uterine tissue of TBH injected group. A significant reduction in endometrial thickness was observed in the uterus tissue of the group that received the hydro-alcoholic extract of ginger associated with TBH injection compared to the control group, receivers of hydro-alcoholic extract of ginger and even TBH injected group.

Key words: oxidative stress, Tert-Butyl hydroperoxidase, hydro-alcoholic extract of ginger, sexual hormones, liver enzymes, total antioxidant capacity, Malondialdehyde, uterus, ovary

INTRODUCTION

Oxidative stress causes physiological changes and inducing of specific metabolic responses with inducing of synthesis of certain antioxidant compounds to control and neutralize free radicals. In poultry industry and animal husbandry, animals are affected by a variety of stresses including heat stress, vocal stress, stresses caused by changes in diet, risk of a variety of diseases, medications, vaccinations, pregnancy and parturition. In the body of animals that are exposed to oxidative stress, free radicals are produced. Oxidative stress is produced in animal's body because of the imbalance between peroxidants and antioxidants (4). This proportion changes due to the increased level of reactive oxygen and nitrogen with reduced antioxidant defensive mechanism (7). Free radicals cause damage to the molecular structures such as DNA, proteins, enzymes and or membrane lipids and thus with increasing free radicals in the body of birds and mammals, the growth rate decreases and their safety is compromised. In animal reproductive physiology, oxidative stress issue and its control by antioxidants is raised. Antioxidants are able to make oxidant agents stable or disable before attacking the cells. Antioxidants can be natural or synthetic and hydro-alcoholic extract of ginger is plant containing natural antioxidant materials. Hydro-alcoholic extract of ginger includes strong antioxidant substances which lead to reduce or prevent the production of free radicals. Ginger extract and its main components have antinausea and anti-vomiting, anti-hepatotoxicity, anti-inflammatory and antioxidant effects and cause gathering and binding free radicals and protection of the cell membrane from oxidation and also reduced lipid peroxidation and increased levels of antioxidant enzymes

(12, 3). Oxidative stress is one of livestock industry problems and is involved in the development of numerous diseases including infection, mastitis, diarrhea, pneumonia and respiratory diseases and zoonoses. Studies on oxidative stress in ruminants is limited and the studies are also scattered and usually are related to the mastitis and retained placenta (16). Adverse effects of oxidative stress on testicular tissue, sperm quality and its function are well documented, but its adverse effects on ovarian tissue, uterus and female sexual hormones have not been fully clarified. Due to the damage caused by oxidative stress in the body of farm animals and the problems encountered in the female reproductive system, it is essential to find an appropriate way to use the antioxidants in animal feed. Antioxidants application is as repellent agents of toxins and also free radicals from the environment of the cells. Antioxidants were studied to reverse and fix damages to the cells and tissues (3). So, the addition of antioxidant compounds to the feed is in order to reduce the damages caused by free radicals and on reproductive hormonal changes. As the hydro-alcoholic extract of ginger is known as a strong antioxidant compound, they can prevent the production of free radicals. New scientific evidences have shown the useful properties of hydro-alcoholic extract of ginger and improvement of disorders after its use (19). Also, the research results show that the hydro-alcoholic extract of ginger can reduce oxidative stress in the brain (17). The purpose of this study is to consider about the protective effects of ginger hydro-alcoholic extract on the created negative effects within oxidation induced by Tert-Butyl hydroperoxide (TBH) on antioxidant and sexual hormones parameters and uterus and ovary histology in the rats that were exposed to oxidative stress.

RESEARCH METHODS

This study was conducted to determine the effect of ginger extract on antioxidant, sexual hormones and uterus and ovary histology parameters of female rats exposed to oxidative stress. Test of the present study was performed in the laboratory complex of Islamic Azad University of Tehran, Sciences and Research Branch from 2014/12/21 to 2015/06/10. The scientific part on mice was performed in the particular room of laboratory animals with all standards including particular cages for mice breeding and specific equipment for feeding and water and also light control equipment, temperature and humidity. 20 female Wistar rats with the average weight of 62±2 g were bought from the Razi institute in Karaj. Mice were kept

separately in cages with the same temperature and in a room with the automatic controlled of day and night (12 hours of light and 12 hours of darkness) and with free access to food and water. 2×2 factorial experiment was conducted in a completely randomized design with 4 treatments and 5 replications. The obtained data was classified using Excel software and analyzed using GLM procedure of SAS software according to the presented statistical model. The comparison of averages was done by Duncan's multiple ranges. 5% confidence level was considered as the significant level. The intended model used in data analysis of the present research is as below:

$$Yijk = \mu + Ti + Pj + TPij + eijk$$

In this model, Yij, μ, Ti, eij, Piand TPij are the observed amount, mean, oxidative stress effect, test error and the reaction between the oxidative stress and ginger extract, respectively.

The mice were weighed after transfer to the animal room. The average weight of each mouse was 62±2 g; then the rats were divided into four groups and each group was consisted of 5 replications. In the present study, the average intake of each calibrator, control and intended samples sets were calculated at first, then we use the semi-logarithmic graph paper to produce the standard permission. A specific piece of uterus and ovaries tissues were removed by scalpel. After passage, tissues were embedded with paraffin. Then samples in paraffin were cut in 5 microns by microtom (Leica20 model, made in Germany). In staining phase and before staining of paraffin sections, paraffin must be taken out and rehydration should also be done. Xylene and Ethyl alcohol with descending grades were used to remove paraffin and rehydration, respectively. After staining, sections need to be dehydrated and transparent. Dehydration and transparency were performed using absolute alcohol and xylene, respectively. A few drops of Canada balsam glue were dropped on the middle of a slide for monting and the coverslip was placed on it to prevent the sample destruction by the probable physical damages.

RESEARCH FINDINGS

By TBH injection, the rate of this index has been increased compared to the control group and a significant difference was also established ($P<0.05$). This increase was similar to Young et al results (2013) with a dose of 0.5 mmol TBH and also with Liu et al results (2002) that the dose was similar to the present experiment. According to Christian et al studies (2012), Malondialdehyde which is a combination of

three main options, is from the basic products of lipid peroxidation and is increased within the stress. So in this study, TBH has creates stress through the increase of Malondialdehyde level.

Receiving of ginger extract compared to the control group caused the decrease of this index (P<0.05).

Finally, TBH injection + receiving the ginger extract caused the increase of this index and also a significant difference compared to the control group (P<0.05), also this group compared to the TBH group is decreased and has a significant difference (p<0.05). As a result, TBH and ginger extract had the greatest and lowest activities and differences, respectively. Malondialdehyde level is increased during the stress, but it is decreased when the ginger extract enters as an antioxidant and our outcome is similar to the results of other researchers. Malondialdehyde is one of the main oxidation products of fatty acids peroxides and thus its increase shows oxidation of the lipids (9).

The total antioxidant amounts including enzymatic and non-enzymatic antioxidants, were decreased by TBH injection compared to the control group and a significant difference has been established too (P<0.05). Receiving the ginger extract has increased this antioxidant compared to the control group and has also established a significant difference (P<0.05). TBH injection + receiving the ginger extract caused the increase in this index compared to the control group and also has not established a significant difference (P>0.05). But it had decrease and increase compared to receivers of ginger extract and TBH group, respectively.

TBH injection caused an increase in this enzyme activity compared to the control group and did not establish a significant difference (P>0.05). Receiving the ginger extract caused decrease in this enzyme activity compared to the control group but did not establish a significant difference (P>0.05). TBH injection + receiving ginger extract caused an increase in this enzyme activity compared to the control group but did not establish a significant difference (P>0.05). Totally, there was no significant difference in the activity of this enzyme (P>0.05). This enzyme had a high activity level within TBH injection but not significant, but its level has been dropped during the receiving of ginger extract.

TBH injection caused an increase in the activity of this enzyme compared to the control group but did not establish a significant difference (P>0.05), but it was significant in Liu et al experiment. Receiving

the ginger extract caused a decrease in the activity of this enzyme compared to the control group but did not establish a significant difference (P>0.05).

TBH injection + receiving the ginger extract caused an increase in the activity of this enzyme compared to the control group but not a significant difference (P>0.05). Also, TBH injection + receiving the ginger extract compared to TBH group caused an increase in the activity of this enzyme that was not significant (P>0.05).

By TBH injection compared to the control group, it is not caused an increase in the activity of this enzyme and establishment of a significant difference (P<0.05). But this increase was significant in the experiment of Hyejin et al (2012). Receiving the ginger extract has been decreased compared to the control group but did not have a significant difference (P>0.05).

TBH injection + receiving the ginger extract caused an increase in the activity of this enzyme compared to the control group as well as receivers of the ginger extract, but did not have a significant difference compared to both groups (P>0.05) but had a significant difference with TBH group (P<0.05). This enzyme had a significant increase during the stress and its activity has been decreased while the ginger extract was received. The interactions between TBH and ginger extract for LH, FSH, estradiol and progesterone hormones was not significant (P>0.05). TBH injection to the rats caused decrease in LH and estradiol (P<0.05) while FSH and progesterone hormones were not affected by this stressing factor (P>0.05). The concentrations of LH and FSH hormones of the rats which received the ginger extract showed increase (P<0.05).

Oxidative stress is created in the body of animal because of the imbalance between peroxidants and antioxidants (4). Disruption is created in this balance due to the increase in the level of reactive oxygen species and or reactive nitrogen and or reducing the defensive mechanism of antioxidants (7). There are many evidences which show that kind of nutrition and specially its antioxidant content and oxidative stress may affect fertility and pregnancy (10). Reactive oxygen species can affect numerous physiologic processes including oocyst maturation, fetal development and pregnancy. A study showed that production of progesterone and estradiol hormones were reduced when hydrogen peroxide was added to the medium containing human luteal cells as a stressing factor (6). In this study which TBH had been used a stressing factor, the concentration of LH and estradiol hormones showed decrease.

The stress due to the TBH may cause to break double bond of phospholipids unsaturated fatty acids, peroxidation and changes in membrane permeability, epithelial destruction and inflammation in uterus and ovary tissues that leads to the imbalance of LH and FSH hormones in the blood through affecting on the specific receptors of these enzymes in hypothalamic-pituitary-gonad axis. A study showed that the feed additive containing a mixture of vitamin E, Fe, Zn, Se and arginine increases the progesterone concentration from 8.2 to 12.8 ng/ml by increasing the antioxidant capacity. In this research, the people who were exposed to the oxidative stress and received antioxidant supplements had higher rates of pregnancy and ovulation (20).

The ginger extract is a plant containing strong antioxidants that leads to reduce or prevent the production of free radicals. The ginger extract and its main ingredients cause collecting and binding of free radicals and protection of the cell membrane from oxidation and also will decrease lipid peroxidation and increase the level of antioxidant enzymes significantly (12). Antioxidants can decrease lipid oxidation process and remove toxins from the cells by neutralizing of free radicals which results in maintaining the biochemical structure of the cells (3). The new scientific evidences have shown the useful properties of ginger extract usand improvement of disorders after using the ginger extract (11). In the present research, receiving the ginger extract by the rats increases certain reproductive hormones. According to the results of this experiment, Farhumand in 2014 reported that use of quercetin as a natural antioxidant compound increased FSH sexual hormone in rats which were exposed to the oxidative stress (1). Also in another research by Kut Abadi in 2014, use of Thymoquinone as a natural antioxidant compound could increase progesterone by reducing oxidative stress (2). It seems that the used natural antioxidant compounds in this research had an important defensive role in inhibition of free radicals and increased the antioxidant defense of cells and finally increase the average of sexual hormones concentrations in female rats.

The results of this research showed that TBH as an oxidative stressing factor reduced the sexual hormones and estradiol which can increase the negative consequences on production of certain sexual hormones and it is likely to have positive effects on reproductive performance.

Uterus tissue structures of different groups were studied with hematoxylin and eosin routine staining by light microscope. In all

groups, epithelial cells of endometrium were covered by simple ciliated and non- ciliated cylindrical cells except TBH group that were inclined to cubic. Endometrium is consisted of uterine complex glands. The endometrial cells are fibroblast and other kinds of resident cells in connective tissue. Uterine glands have simple cylindrical lining cells similar to uterus surface. Basal layer is observed as a thin clinging layer to myometrium. Myometrium is formed from two thick inner annular and thin longitudinal external muscle layers that the connective tissue associated with large blood vessels were seen between two layers. Perimetrium which is the outermost layer contains connective tissue with a thin serous layer and plenty of lymph vessels.

In pathology study, any endometrial hyperplasia, bleeding and or change in epithelial tissue of endometrium and in the thickness of each three layers of endometrium, myometrium and Perimetrium were not seen in the prepared slides of the control group compared to the normal uteruses.

In pathology study of prepared slides from the receivers of ginger extract, a significant increase in the thickness of endometrium layer of tissues was observed.

In pathology study of the prepared slides from TBH injection group, any endometrial hyperplasia and bleeding were not seen, but the issue which draws the attention more than anything is changes in the structure and a significant decrease specially in the thickness of endometrium in this tissue. Also, changes in endometrial epithelial cells as a transformation from simple ciliated and non-ciliated cylindrical cells to short cylindrical and even cubic cells was seen.

In pathology study of prepared slides from the receiver group of ginger extract associated with TBH injection, any endometrial hyperplasia was not seen but the thickness of endometrium and myometrium layers were lower compared to the control group and the receiver group of ginger extract and even TBH injection group, however the thickness variation of myometrium layer was less in diameter.

In the ovary of the control group that was received 0.2 ml of olive oil, some mature follicles and also some corpus luteum with growing follicles were seen. There are 13 growing follicles, 1 mature follicle as well as 5 corpus luteum. No impact of apoptosis was seen in this group.

In the ovary of receiver group of ginger extract, the primary and secondary growing follicles with a little corpus luteum were

seen. Mature follicle was observed in this group. There was no significant lesion in the ovary of rats that were received ginger extract. The ovary of this group had 8 growing follicles and 4 corpus luteum.

In the ovary of TBH injection group, a significant number of corpus luteum were seen as well as growing and mature follicles. A considerable injury was not observed in the ovary of this group except the apoptosis that was seen only in one of growing follicles. The ovary of this group had 17, 2 and 6 growing and mature follicles and corpus luteum, respectively.

In ovary of the group who had received ginger extract and TBH injection together, significant numbers of corpus luteum were seen. Mature follicles were not observed in the ovary of this group while growing follicles were seen. Ovary of these rats had 8 growing follicles, 1 mature follicle and 3 corpus luteum. In a growing follicle of granulosa cells, apoptosis was seen at the second growing stage but there was no difference with TBH injection group from the intensity viewpoint.

In Parhizkar et al experiment (2011) that the effect of buckwheat was considered as an antioxidant, buckwheat increased the vaginal leucocytes. Also, in the experiment of Liu et al (2000) hydrogen peroxide which is the cause of oxidative stress like Tert-Butyl Hydroperoxide in the laboratory studies caused to stop the growth of oocyst and induce the cell death.

DISCUSSION AND CONCLUSION

TBH caused an increase in Alkaline phosphatase activity and Malondialdehyde level and decrease in the total antioxidant capacity that shows the creation of oxidative stress and weakness of antioxidant system. TBH also reduced the amounts of sexual hormones compared to the normal condition (control group) and thus this decrease may reduce the performance of female immune system. In histology study, TBH also caused some tissue injuries including hydropic degeneration in stroma and follicular cells and increase in eosinophil penetration to ovary tissue and hydropic degeneration and increase in eosinophil penetration in uterus tissue that indicated oxidative stress injury in the reproductive system of female rat. Ginger extract with its antioxidant properties caused decrease in ALP activity and MDA level and increase in total antioxidant capacity that indicates the improvement of antioxidant system situation of the body. On the other hand, ginger extract increased progesterone hormone to normal and thus improved the reproductive function. But in histology, ginger extract not only not able to remove the

adverse effects of TBH but also it increased some tissue injuries in uterus besides TBH that it can be due to high dose of ginger extract and a decrease in dose and duration of treatment with ginger extract may prevent these complications.

Evaluation Of Antioxidant Parameters			
Factors Assessed	**ALP(Unit L)**	**AST(Unit L)**	**ALT(Unit L)**
Main Effect			
TBHInjection	**64/96**	**54/93**	**33/85**
Lack Of TBH Injection	**56/65**	**47/53**	**25/48**
Ginger Extract	**58/15**	**48/64**	**28/33**
Lack Of Ginger Extract	**63/46**	**53/85**	**31/00**
Interaction Effect			
Control	**58/66 ab**	**50/26 a**	**26/23 ab**
Injection TBH - Lack Of Ginger Extract	**68/26 a**	**43/57 a**	**35/76 a**
Lack Of TBH Injection - Ginger Extract	**54/63 b**	**44/80 a**	**24/73 b**
Injection TBH – Ginger Extract	**61/66 ab**	**52/43 a**	**31/93 ab**
SEM	**3/91**	**3/77**	**3/07**
TBH P-Value	**0/066**	**0/085**	**0/026**
Ginger Extract P-Value	**0/211**	**0/203**	**0/411**
P-Value TBH * Ginger Extract	**0/751**	**0/952**	**0/714**

Evaluation Of Liver Enzymes		
Factors Assessed	**MDA (Micromoles Per Liter)**	**TAC(Percent Free Radical Scavenging)**
Main Effect		
TBHInjection	**6/97**	**21/65**
Lack Of TBH Injection	**4/39**	**30/32**
Ginger Extract	**4/71**	**30/92**
Lack Of Ginger Extract	**6/64**	**21/05**
Interaction Effect		
Control	**4/710 b**	**24/11 b**
Injection TBH - Lack Of Ginger Extract	**8/580 a**	**17/99 b**
Lack Of TBH Injection - Ginger Extract	**4/070 b**	**36/53 a**
Injection TBH – Ginger Extract	**5/363 b**	**25/31 b**
SEM	**0/443**	**2/56**
TBH P-Value	**0/0004**	**0/009**
Ginger Extract P-Value	**0/002**	**0/004**
P-Value TBH * Ginger Extract	**0/019**	**0/349**

Evaluation Of Sexual Hormones

Factors Assessed	**P4(Ng Per ml)**	**ES (Pico Per ml)**	**FSH(Ng Per ml)**	**LH(Ng Per ml)**
Main Effect				
Injection TBH	**8/86 a**	**14/317 b**	**435/33 a**	**30/983 b**
Lack Of TBH Injection	**9/45 a**	**18/25 a**	**475/83 a**	**37/200 a**
Ginger Extract	**9/71 a**	**17/467 a**	**484/67 a**	**37/067 a**
Lack Of Ginger Extract	**8/60 a**	**15/100 a**	**426/50 a**	**31/117 a**
Interaction Effec				
Control	**9/040 a**	**16/76 ab**	**435/0**	**34/46 ab**
Lack Of Ginger Extract - Injection TBH	**8/166 a**	**13/43 b**	**418/0**	**27/76 b**
Ginger Extract - Lack Of TBH Injection	**9/860 a**	**19/73 a**	**516/6**	**39/93 a**
Ginger Extract–Injection TBH	**9/560 a**	**15/20 ab**	**452/56**	**34/20 ab**
SEM	**0/747**	**1/63**	**31/00**	**2/62**
TBHP-Value	**0/454**	**0/043**	**0/227**	**0/045**
Ginger Extract P-Value	**0/176**	**0/186**	**0/097**	**0/050**

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