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Linalool Improve Biochemical Damage and Fatty Acids Composition of Testes on Fasting Male Rats

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Abstract: The aim of this study was to evaluate fatty acids compositions, oxidative stress levels and some antioksidant levels in the testes of 24 h fasted rats and linalool application. Adult male Wistar albino rats were assigned as Control (C), 24 h fasted (24 h F) group and added linalool (120 mg kg^{-1}) during 24 h fasted (24 h F+L) groups. After applications, the animals were killed and the testes were excised for determination of tissue malondialdehyde, Glutation Peroxidase (GSH-Px), the activity of superoxide dismutase and determination of tissue fatty acids compositions. The activites of Superoxide Dismutase (SOD), Glutathione peroxidase (GSH-Px) and the level of Malondialdehyde (MDA) in testicular tissue specimens were determined spectrophotometrically. The fatty acid compositons in the testes were analyzed as percent by gas chromatography (GC). Researchers calculated between C16:0 (palmitic acid) and C22:6 (docosahexaenoic acid) fatty acids in the testes of all experimental groups. Treatment with fasted rats resulted in a significant reduction in C16:1 (palmitoleate) fatty acids when compared to the control rats ($p<0.05$). C16:1 fatty acids were higher in linalool application than fasted groups ($p<0.05$). C22:4 (docosatetraenoic Acid), C22:5 (docosapentaenoic acid), C22:6 n3 (docosahexaenoic acid) fatty acids were lower in the fasted group than the control group. In addition, C22:4, C22:5, C22:6 fatty acids compositions in the linalool treatment group were higher than the fasted group ($p<0.05$). Saturated fatty acids levels in all experiment groups were not change as statistically ($p>0.05$). SOD and GSH-Px levels in the fasted rats were statistically lower than control groups ($p<0.05$). Both SOD and GSH-Px levels in the linalool application groups were not statistically a change compared to fasted groups ($p>0.05$). In addition, MDA levels in the fasted groups were higher than control groups. Besides, MDA levels in the linalool application groups were lower compared to fasted groups ($p<0.05$). These findings indicate that fasted state has oxidative effects on testicular tissue and linalool has protective effects on male reproductive system.

Key words: Linalool, fasting, testes, rat, fatty acids, SOD, MDA, GSHPx, oxidative stress

INTRODUCTION

Linalool is a fragrance ingredient used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its worldwide use is in the region >1000 metric tonnes per annum (Lapczynski *et al*., 2008; Bickers *et al*., 2003). Linalool, a monoterpene alcohol is also present in essential oils of many medicinal and aromatic plants that are endowed with many biological activities, including antioxidant, antimicrobial, antiviral, anti-inflammatory and antitumor properties (Tepe *et al*., 2004; Pattnaik *et al*., 1997; Mitic-Culafic *et al*., 2009; Koroch *et al*., 2007; Celik and Ozkaya, 2002). Besides, Linalool possesses several depressant effects on the central nervous system and some finding is important in showing the potential usefulness of linalool as a pharmacotherapeutic agent (Leal-Cardoso *et al*., 2010).

Fasting states occur oxidative stres in some animal tissue. In several studies, lipid peroxidation, nitric oxide synthase activity and hydrogen peroxide accumulation have been reported as a result of fasted. Some researchers have indicated that total Glutathione (GSH), mitochondrial GSH and the activities of major antioxidant enzymes were lower in the fasted mice (Abdelmegeed *et al*., 2009; Brown *et al*., 1995). To protect the integrity of biological membranes from detrimental oxidative processes caused by free radicals both enzymatic and non-enzymatic defense mechanisms are present in the cell (Droge, 2002;

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Kara *et al*., 2007). All animal cells have intense phospholipid layer in both cell membrane and organelle membrane. These phospholipids contain PUFA (Polyunsaturated Fatty Acids) and they are directly effected by reactive oxygen species (Bourre *et al*., 1992; Halliwell, 1994). α-linolenik asit, Eicosapentaenoic Acid (EPA) and DocosahExaenoic acid (DHA) are known as omega-3 fatty acids and these fatty acids levels are effected by oxidative stress state (Lonergan *et al*., 2002). Testes which are the source of fertility and heredity in male mammals are particularly sensitive feeding (Santos *et al*., 2004; Conn, 1986). Secretion of testosterone is impaired due to excessive oxidative stress and the degeneration of leydig cells (Santos *et al*., 2004; Bandyopadhyay *et al*., 1999). Lipid peroxidation that is caused by reduced oxygen directly effect the spermatozoon membrane where contain pollyunsaterated fatty acids (Sikka, 1996). In this study, Researchers investigated the fatty acids changes, some antioksidant enzyme and lipit peroxidation both fasting state and the linalool application to fasting on rat testes.

MATERIALS AND METHODS

Animals and experimental application groups: In this study, 24 adult male Wistar albino (Firat University Experimental Research Center, Turkey) rats at 12 weeks of age were used (in the each group, n = 8/group). The animals were group housed in cages in a climate-controlled room with a 12 h light/12 h dark cycle and in room temperature (20±3°C) has been provided. Animals which have been used in the study are obtained from Elazig-Turkey Animal Health and Research Institute. During experiment, criteria which are determined by NIH (National Institutes of Health) are obeyed conscientiously as related with animal rights. Animals were randomly divided into three groups. The first group consisted of normally-fed controls, the second group was fasted for 24 h, the third group was fasted for 24 h+linalool (120 mg kg^{-1}). Drinking water was given to fasting animal groups *ad libitum*. Rats in the second group were not given any food for 24 h while rats in the third group were given linalool during 24 h fasting. Linalool was given at the first 12 h fasting. At the end of the experiment, the animals were anesthetized by intramuscular (i.m.) injection of ketamine (70 mg kg^{-1}) (Ketalar, Parke-Davis, Eczacibasi, Istanbul, Turkey) and xylazine (5 mg kg^{-1}) (Rompun, Bayer, Istanbul, Turkey) and blood was drawn from the heart by cardiac puncture and the investigated testes tissues were removed from animals and frozen until analyzed.

Chemicals: Linalool were purchased from Sigma (St. Louis, MO). All the other chemicals used in the study were of analytical grade and were purchased from Merck (Darmstad, Germany).

Extraction of lipids and preparation of fatty acid methyl esters: The lipids of testes tissue samples were extracted by the method of Hara and Radin (Hara and Radin, 1978). Testes tissue samples (0.5 g) were homogenized in 5 mL hexane/isopropanol mixture at 3:2 (v/v) for 30 sec. Samples were centrifuged at 4500 rpm for 10 min; supernatants were transfered to covered tubes and fatty acids of the lipid extract were converted to methyl esters by using 2% sulfuric acid (v/v) in methanol (Christie, 1992). Fatty acid methyl ester forms were extracted with n-hexane. Analysis was performed in a Shimadzu GC-17A V3 (Kyoto, Japan) intstrument gas chromatograph equipped with a Flame Ionization Detector (FID) and a 25 m, 0.25 mm i.d. Permabond fused-silica capillary column (Macherey-Nagel, Germany). The oven temperature was programmed between 120-220°C, 5°C min^{-1}. Injector and FID temperatures were 240 and 280°C, respectively. The nitrogen carrier gas flow was 1 mL min^{-1}. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. Class GC 10 software version 2.01 (Shimadzu, Kyoto, Japan) was used to process the data. The results were expressed as percent.

Determination of superoxide dismutase activity: Total SOD activity was determined by the method of Sun *et al*. (1988). The technique is based on inhibition of Nitro Blue Tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as the superoxide generator. The SOD activity was measured in the ethanol phase of the supernatant after 1 mL of ethanol-chloroform mixture (5:3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as U g^{-1} protein.

Glutathione peroxidase (GSH-Px) activity: GSH-Px activity was evaluated by the Flohe and Gunzler method (Flohe and Gunzler, 1984; Tasset *et al*., 2008). The testes tissues were homogened in ice-cold buffer (0.1 M KH_2PO_4/K_2HPO_4, pH 7.0 plus 29.2 mg EDTA in 100 mL of distilled water and 10.0 mg digitonin in 100 mL of distilled water, final volume, 2000 mL) to produce a homogenate. The homogenates were then centrifuged at 10,000×g for 10 min at 4°C. The GSH-Px assay is based on the oxidation of NADPH to NAD^+, catalyzed by a limiting concentration of glutathione reductase with maximum abasorbance at

340 nm. The activity of GSH-Px is expressed as units per milligram of protein (U mg^{-1} protein) and absorbance was evaluated in a spectrophotometer (UV-1603 Shimadzu, Kyoto, Japan).

Determination of tissue Malondialdehide (MDA) levels: Esterbauer method that is lipid peroxidation measurement method was used to determination of tissue malondialdehide levels (Esterbauer and Cheeseman, 1990). Tiobarbituric acid which is reacting with MDA at 90-95°C was used in order to occur pink kromogen. Then tissue samles was cooled for 30 min and absorbances was read as spectrophotometrical at 532 nm. All results was calculated as nmol g^{-1} tissue protein.

Statistical analysis: The SPSS software for Windows, v.10 (SPSS, Chicago, IL) was used for the statistical treatment of the data. The results are expressed as mean values±standard deviation. Differences between means were established by analysis of variance with $p<0.05$ considered significant. Significant differences among treatment and control groups were interpreted using the Tukey's honest significant difference post-hoc test.

RESULTS

The present study examined the MDA, SOD, GSH-Px and fatty acid composition on testes of Wistar rats which were administered with normal fed, fasted state and linalool aplications. The results were shown in Table 1 and Fig. 1-3.

Table 1: Fatty acid composition of rat testes (%/g); a) $p<0.05$ significant, compared to control; b) $p<0.05$ significant, compared to 24 h F

Fatty acids	Control	24 h F	24 h F+L
C16:0	23.82±1.52	24.45±1.37	22.41±1.74
C16:1 n7	4.98±1.16	2.46±0.54[a]	3.68±0.42[ab]
C17:0	0.41±1.02	0.37±0.12	0.36±0.05
C18:0	5.40±0.48	5.98±0.52	6.02±1.23
C18:1 n9	16.78±1.56	15.43±1.43	15.23±1.42
C18:1 n11	4.68±1.23	4.21±0.12	4.34±0.19
C18:2 n6	12.18±1.24	11.98±1.15	12.14±1.32
C18:3 n6	1.35±0.22	1.45±0.17	1.22±0.21
C20:1 n9	0.59±0.21	0.64±0.14	0.49±0.02
C20:2 n6	0.42±0.84	0.47±0.04	0.43±0.16
C20:3 n6	0.84±0.12	0.90±0.06	0.89±0.18
C20:4 n6	12.10±1.54	13.02±1.23	13.42±0.34
C22:2 n6	0.43±0.17	0.50±0.08	0.46±0.46
C22:4 n6	1.18±0.14	0.65±0.18[a]	1.05±0.52[b]
C22:5 n6	12.65±1.23	8.47±1.58[a]	10.94±1.22[ab]
C22:5 n3	0.47±0.04	0.51±0.05	0.49±0.07
C22:6 n3	1.08±0.15	0.62±0.06[a]	1.24±0.16[b]
∑Saturated	29.63±0.20	30.80±0.54	28.79±1.08
∑Unsaturated	72.73±1.23	61.31±1.06[a]	66.02±1.17[ab]
∑MUFA	26.44±0.12	22.74±1.10	23.51±1.22
∑PUFA	46.29±1.02	38.57±1.23[a]	42.51±1.09[b]
Satur./unsatur.	0.40±0.03	0.50±0.02	0.43±0.07

Fatty acid composition of rat testes on experimental groups: The results of the different fatty acids values are shown in Table 1. The saturated fatty acids which are C16:0, C17:0 and C18:0 fatty acids composition were not statistical significant among all experimental groups ($p>0.05$). Adversely, the monounsaturated fatty acids like C16:1 were lower compared to the control group. Likewise, polyunsaturated fatty acids like C22:4, C22:5 C22:6 in the fasted groups were lower compared to control group. Both monounsaturated fatty acids and polyunsaturated fatty acids compositions in the linalool aplication groups were higher than fasted groups ($p<0.05$). C22:6 n3 and C22:4 n6 fatty acids levels in the linalool aplication groups were not statisticaly significant compared to control ($p>0.05$).

The activites of Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and the level of Malondialdehyde (MDA) in testicular tissues: The results indicate that the GSH-Px level decreased in the both fasted group and linalool application group compared to control. Similarly, SOD levels were lower in the both fasted and linalool application group compared to control ($p<0.05$). Both SOD and GSH-Px levels in linalool

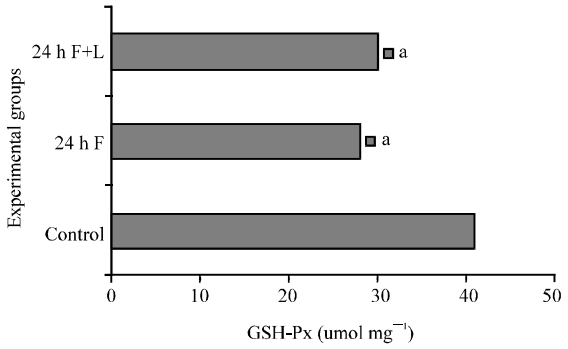

Fig. 1: The levels of GSH-Px in the testes; a) $p<0.05$ significant compared to control

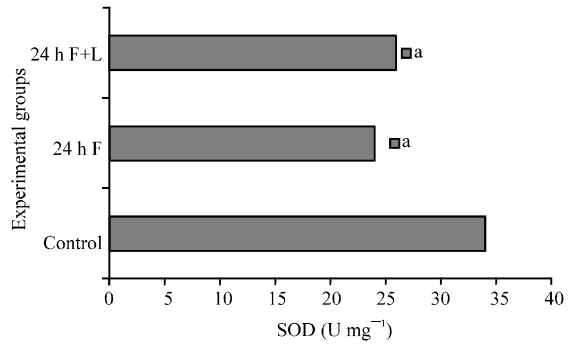

Fig. 2: The levels of SOD in the testes; a) $p<0.05$ significant compared to control

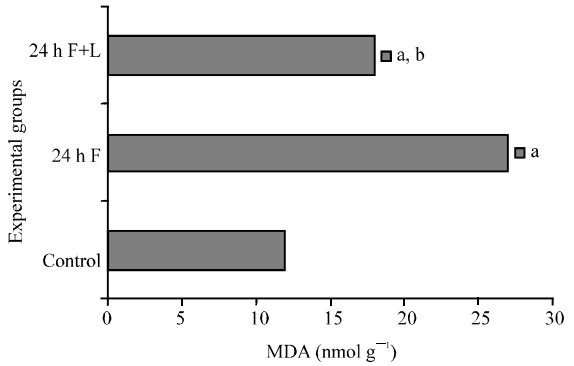

Fig. 3: The levels of MDA in the testes; a) $p<0.05$ significant compared to control; b) $p<0.05$ significant compared to 24 h F

application were not statisitical significant compared to fasted group ($p>0.05$, Fig. 1 and 2). Besides, the the finding indicate that MDA level increased in the fasted group compared to control group. Diametrically, the MDA level in the linalool group was lower as statisticaly than the fasted group ($p<0.05$, Fig. 3).

DISCUSSION

Fasting state or caloric restriction effect testicular cell metabolism, sex behavior and rogen receptors and testosterone concentration (Bandyopadhyay *et al*., 1999). Liver glycogen stores are virtually depleted within 24 h (Rothman *et al*., 1991). As liver glycogen stores are virtually depleted within 24 h, gluconeogenesis supplies the requirements of the brain and other glucose-requiring tissues. Falling insulin concentrations lead to both net proteolysis in muscle with release of alanine and glutamine and lipolysis in adipose tissue with release of glycerol and Non-Esterified Fatty Acids (NEFAs) (Nygren, 2006; Awad *et al*., 2009). The oxidative stres in testicular milieu is associated with DNA damage, lipid peroxidation and produces higher frequency of abnormal sperms with significant effect on male fertility (Kumar *et al*., 2002). Under normal physiological conditions, free radicals are generated in testis subcellular compartments, particularly mitochondria which are subsequently scavenged by antioxidant defense systems of the corresponding cellular compartments (Bergendi *et al*., 1999). Further, the mitochondrial membrane is more susceptible to Lipid Peroxidation (LPO) as this compartment is rich in polyunsaturated fatty acids and has been shown to contain low amounts of antioxidants (Selvakumar *et al*., 2005). Adversely, polyunsaturated fatty acids levels were calculated as low level by Gavazza and Catala (2003) in rat testes during Fe^{+2}-iduced oxidative stres (Gavazza and Catala, 2003). In paralel in the study, total Polyunsaturated Fatty Acid (PUFA) levels were low rate on fasting groups compared to controls. Particularly, C22:4 n6 (Docosatetraenoic Acid), C22:5 n6 (Eicosapentaenoic Acid) and C22:6 n3 (Decosahexaenoic Acid) fatty acids were low in fasting groups compared to control. We also calculated that total PUFA levels were high in linalool groups compared to fasting group. PUFAs of linalool application were not statistically significant difference compared to control ($p>0.05$, Table 1). Abul *et al*. (2002) expressed that membranes lipids destruction made with aldehydes occurs endoperoksit (Abul *et al*., 2002; Rosenblum *et al*. (1989) also indacted that lipids peroxidations were occured by reactive oxygen species (Rosenblum *et al*., 1989). In the study, malondialdehyde that is marker of oxidative stress were high in fasting groups and GSH-Px, SOD that are antioxidant enzyme in fasting groups were low compared to control (Fig. 2 and 3). Linalool applications improved MDA levels. That is MDA level in the linalool groups was low level compared to fasting groups ($p<0.05$, Fig. 3). Fasting state or starvation effect some hormone levels in the rat tissues. Hormones such as insulin, glucagon, leptin, ghrelin and the gonadal hormones determine energy partitioning that is they direct metabolic fuels into or out of storage (Schneider and Watts, 2009; Wells, 2009). Especially, ghrelin peptide that is the endogenous ligand for the Growth Hormone (GH) Secretagogue Receptor (GHS-R) increase during fasting state (Kojima and Kangawa, 2005; Kojima *et al*., 1999). The ghrelin gene is expressed in stomach, small intestine, brain, pituitary, salivary gland, adrenal, ovary and testis with maximum expression occurring in the stomach (Ghelardoni *et al*., 2006; Aydin *et al*., 2005). It has recently demonstrated the ability of ghrelin has anti-proliferative effects on different testicular cell types and is a negative modulator of male reproductive system (Kheradmand *et al*., 2009). Ghrelin effects to fatty acid synthesis and related transcription factor mRNA levels (Buyse *et al*., 2009; Muccioli *et al*., 2004). Lopez *et al*. (2008) expressed that fasting state reduced malonyl-CoA and fatty acid synthase levels (Lopez *et al*., 2008). Theander-Carrillo *et al*. (2006) reported that ghrelin action in the brain controls adipocyte metabolism (Theander-Carrillo *et al*., 2006). The Stearoyl-CoA Desaturases (SCD) are important on unsaturated fatty acids metabolism in animal tissues. SCD can act on chain lengths from C12:0-C19:0 (Mauvoisin and Mounier, 2011; Zolfaghari and Ross, 2003). However, SCD1 preferred desaturation substrates are palmitoyl-CoA (C16:0) and stearoyl-CoA (C:18:0) which are converted to palmitoleoyl-CoA (C16:1) and oleoyl-CoA (C18:1)

respectively (Flowers and Ntambi, 2008). Ambati *et al.* (2010) reported that in rat, injection of ghrelin decreases the level of Stearoyl-CoA Desaturase 1 (SCD1) mRNA in adipose tissue (Ambati *et al.*, 2010). In parallel, we determined that C16:1 was lower in the 24 h F groups than control ($p<0.05$). Many researcher stated that no histopathologic abnormalities were observed in animals in the high-dose linolool in the liver, adrenals, brain, heart, kidneys, thyroids, mesenteric lymph node, spinal cord, testes, ovaries, spleen, urinary bladder, sternal bone marrow, pituitaries or sciatic nevre (Letizia *et al.*, 2003; Tepe *et al.*, 2004; Pattnaik *et al.*, 1997; Mitic-Culafic *et al.*, 2009). The results of the study are consistent with these reports, demonstrating that linalool effects MDA levels that is indicator for lipid peroksidation. At the same time we determined that linalool protected both monounsaturated and polyunsaturated fatty acids levels (Table 1). Researchers also determined that both C16:1 C22:6 fatty acids were higher as statistically in 24 h F+Linalool groups than 24 h F groups ($p<0.05$).

CONCLUSION

In the study, we suggest that linalool was a potential candidate in combating the testicular abnormalities induced by fasted. According to results of the study, the linalool can protect polyunsaturated fatty acids composition in the rat testes against to fasted state. Although the exact mechanisms remain to be clarified, linalool could be an protective effects to testicular cell metabolism.

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