Effect of Alcohol on the Changes of Testicular Protein Expression in Adult Male Rat

Presented by
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Ethanol (alcohol)

- An organic compound

![Chemical structure of ethanol](image)

Entertainments

Life problems

Reactive oxygen species (ROS)

DNA damage

Mitochondria dysfunction

Protein adduct

lipid peroxidation
Effects of alcohol on male reproductive system in animals

- **Testicular weight**
  - Adaramoye and Arisekola, 2013
  - Akbari et al., 2017
  - Nishi et al., 2018

- **FSH, LH, & Testosterone level**
  - Akbari et al., 2017
  - Yari et al., 2018
  - Nishi et al., 2018

- **Diameter of seminiferous tubule**
  - Uygur et al., 2013
  - Mesquita et al., 2013
  - Yari et al., 2018

- **Sperm quality**
  - Sperm concentration
  - Normal sperm morphology
  - Sperm motility
  - Sperm viability
  - Siervo et al., 2015
  - Akomolafe et al., 2016
  - Nishi et al., 2018
  - Yari et al., 2018

- **Acrosome reaction**
  - Roger et al., 1987

- **Changing of seminiferous tubule histology**
  - Vacuolization
  - Loss of germ cell
  - Germ cells detachment
  - Oremosu et al., 2013
  - Abarikwu et al., 2016
  - Akomolafe et al., 2016
  - Yari et al., 2018
  - Nishi et al., 2018

- **Germ cell apoptosis**
  - Zhu et al., 2000
  - Kok and Kim, 2006
  - Uygur et al., 2013

- **17βHSD and 3βHSD activity**
  - Akomolafe et al., 2016
Tyrosine phosphorylated (TyrPho) proteins present in male reproductive organs and essential for male fertility

TyrPho proteins are involved in spermatogenesis and testosterone synthesis (Chaichun et al., 2017; Sawatpanich et al., 2018; Tongpan et al., 2019).

**Testis**
- Sertoli cells
- Spermatogonia
- Round and elongated spermatids
- Leydig cells (Chaichun et al., 2017)

**Epididymis**
- Cytoplasmic principle cells
- Nuclei of apical & basal cells
- Sperm mass (Sawatpanich et al., 2018)

**Seminal vesicle**
- Seminal epithelium and fluid (Tongpan et al., 2019)
TyrPho protein expressions are changed in testis induced with many infertility substances

- **Mimosine**
  (Burawat et al., 2018; Chaichun et al., 2019)

- **Valpoic acid**
  (Sukhorum and Iamsaard, 2017; Tongpan et al., 2019)

- **Ketoconazole**
  (Iamsaard et al., 2014)

- **Methotrexate (MTX)**
  (Iamsaard et al., 2018)

- **Type I and II diabetes**
  (Sampannang et al., 2019; Yannasithinon and Iamsaard, 2019)

- **Acute and chronic stresses**
  (Arun et al., 2016a; b)

- **Alcoholic animal model**
  (This study)

- **Alteration of TyrPho protein**

  - Sperm quality
  - Testosterone level
  - Testicular damage
Objectives

To investigate the effect of alcohol on changes of testicular tyrosine phosphorylated protein expression in rats.
Research Methodology

Animal Ethics Committee of the Faculty of Medicine, Khon Kaen University
(AEMDKKU 009/2019)
Experimental design

(Yari et al., 2017)

Adult male Wistar rats
(weighing between 200-250 g, n = 14)

Control (Con)

Distilled water at 4 ml/day, p.o.

Alcohol (Alc)

Ethanol at 5 g/kgBW/day (40% v/v), p.o.

14 consecutive days (2 weeks)
Weighed & terminated

Thiopental sodium 80 mg/kg BW, IP

Serum testosterone level

Epididymis & vas deferens

Right testis

Morphological study by hematoxylin & eosin staining

Left testis

Testicular protein expression by immuno Western blot analysis

Squeezed

- Sperm concentration
- Sperm morphology
Results
No difference of reproductive organ morphology in alcohol treated rats

Alcohol significantly decreased BW but increased relative weights of testis and epididymis plus vas deferens

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Alcohol</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of increased body weight (BW)</td>
<td>46.535±2.509</td>
<td>31.128±8.097*</td>
<td>0.02</td>
</tr>
<tr>
<td>Absolute testis weight (g)</td>
<td>1.647±0.075</td>
<td>1.694±0.099</td>
<td>0.30</td>
</tr>
<tr>
<td>Relative testis weight (g/100gBW)</td>
<td>0.528±0.020</td>
<td>0.609±0.033*</td>
<td>0.00</td>
</tr>
<tr>
<td>Absolute epididymis + vas deferens weight (g)</td>
<td>0.461±0.012</td>
<td>0.481±0.033</td>
<td>0.35</td>
</tr>
<tr>
<td>Relative epididymis + vas deferens weight (g/100gBW)</td>
<td>0.148±0.012</td>
<td>1.164±0.017*</td>
<td>0.00</td>
</tr>
<tr>
<td>Absolute seminal vesicle + prostate gland weight (g)</td>
<td>1.319±0.188</td>
<td>1.276±0.163</td>
<td>0.76</td>
</tr>
<tr>
<td>Relative seminal vesicle + prostate gland weight (g/100gBW)</td>
<td>0.423±0.051</td>
<td>0.459±0.066</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* Significant difference (p < 0.05) compared to the control
Abnormal sperm morphology found in alcoholic rats

Normal sperm

Head and tail abnormal sperm

- Banana head
- Bent neck
- Amorphous head
- Tailless

Multiple abnormal sperm

- Bent tail
- Broken tail
- Headless
- Spiral tail

Alcohol significantly increased sperm abnormality

* Significant difference (p < 0.05) as compared to the control
Alcohol induced some seminiferous atrophies

Sloughing germ cells and vacuolization of seminiferous epithelium observed in alcoholic rats
Alcohol did not change sperm concentration but decreased serum testosterone levels.

Alcohol increased StAR and HSP70 but decreased AR expressions in testicular tissue.
Significantly, expressions of TyrPho proteins decreased in alcohol-treated rat testis.

* Significant difference (p < 0.05) compared to the control
Discussions
Relative weight of testis & epididymis plus vas deferens
(Uygur et al., 2014)

Body weight
(Uygur et al., 2014)

Serum testosterone level
(Yari et al., 2018)

Induce

Androgen receptor (AR)
(O’Hara & Smith, 2015; Horibe et al., 2019)

Lipid peroxidation

Testicular histopathology
- Seminiferous atrophies
- Sloughing germ cells
- Vacuolization of seminiferous epithelium
(Uygur et al., 2014; Akang et al., 2015; Siervo et al., 2015)

Abnormal sperm morphology
(Siervo et al., 2015)
Heat shock protein 70 (HSP70)
(Normal spermatogenesis & cell protection)
(Akagi et al., 2012; Kamal & Omran, 2013; Mobaraki et al., 2017)

Androgen receptor (AR)
(Spermiation and germ cell maturation and survival)
(O’Hara & Smith, 2015; Horibe et al., 2019)

Abnormal sperm morphology
(Siervo et al., 2015; da Silva et al., 2017)

Protection of spermatogenesis
(Akagi et al., 2012)

Testicular histopathology in alcoholic rats

Spermatogenesis

Normal sperm

(Meehan & Sadar, 2003)
Steroidogenic acute regulatory protein (StAR)
(Transfer of cytosolic cholesterol to mitochondrial inner membrane for testosterone synthesis)
(Kim et al., 2003)

Maintain the homeostasis of hypothalamic-pituitary-gonadal axis
**Expressions of TyrPho proteins**
(spermatogenesis and testosterone synthesis)
(Arun et al., 2016)

**StAR expression**

**Serum testosterone level**

**AR expression**

**Abnormal sperm morphology**
Conclusion

Alcohol

Changes

Testicular AR, TyrPho, HSP70, and StAR expressions

induce

Sperm and testis abnormalities
- sperm morphology
- Testicular histopathology

Male infertility.
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Thank you for your attention