**Male Rat Susceptibility for Liver and Kidney Injury**

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Abstract—The experimental study of this paper was designed to investigate male rat susceptibility to liver injury. A combination of two experimental animal models (Lead acetate for tissue injury (80 mg/L) and castration) had been used on twenty male rats, they were divided into two groups sham (n = 10); castrated (n = 10). Results revealed that; liver weight reduced significantly (P < 0.05) in sham group in comparison with castrated rats, but kidney weight changed slightly. Also, serum aminotransferase (AST) was significantly higher in sham versus castrated rats. Neither alanine aminotransferase (ALT) and alkaline phosphatase (ALP) nor malondialdehyde (MDA) changed. In conclusion, the absence of male sex hormone would delay tissue injury of male rat organs especially liver organ.

Index Terms—Alanine aminotransferase, liver injury, testosterone.

I. INTRODUCTION

Lead acetate is widely used in experimental animals for pathophysiological studies (Venkareddy and Muralidhara, 2015), because it plays an important role in hepatotoxicity and lipid peroxidation (Sharma, et al., 2015). Castration is a process involves removing male tests (Fan, et al., 2014), and Lead acetate causes liver injury, a combination between them generates changes in liver and kidney weights, lipid peroxidation, and liver enzymatic activity.

Although, liver is the site of production most of the antioxidant enzymes and purifies body from all toxins and body waste substances (Shymans'kyi, et al., 2014), kidney also has a crucial role in purification and regulation of body fluid balance (Gueutin, et al., 2013), their size and weight of organs are reflection of their physiological actions, a minor change of them is taken in medical consideration. Either an increase or decrease in anatomy and physiology of liver and kidneys produce problem for the whole body (Block, et al., 2015; Vilar-Gomez, et al., 2015).

On the other hand, lipid such as cholesterol is a precursor for many steroidal hormones such as testosterone, which contributes in male sex organ development and liver cell proliferation (Kelly, et al., 2014). Also, lipid reacts with free radicals to produce lipid peroxide (Perez-Rodriguez, et al., 2015); malondialdehyde is a byproduct lipid oxidation, usually it is used to detect free radical injury indirectly through reaction with thiobarbituric acid, commonly named TBA-MDA reaction (Papastergiadis, et al., 2012).

Measurement of serum liver enzymatic activity changes, called liver function tests (LFT) includes (AST), (ALT), and (ALP) (Mikolasevic, et al., 2015), their concentration in the plasma fluctuate in proportion to physiological events of liver and heart, either in normal or in abnormal situations (Naik, et al., 2011). Therefore, in most experimental animal models especially inducing liver injury, the LFT is the well-known parameter to measure activity of liver enzyme (Wang, et al., 2008).

The fluctuation of hormonal concentration has negative consequences on the liver tissue architecture, lipid oxidation, and enzymatic activity (Naik, et al., 2011; Kelly, et al., 2014; Pertsov, et al., 2014), but until now the reason behind male vulnerability to liver injury is not fully understood. So the present study was designed to investigate mysterious effects of male sex hormone on liver and kidney damage, by using a combination animal model, lead acetate to produce organ injury and castration to inhibit testosterone effects on the body.

II. MATERIALS AND METHODS

A. Animals

The present study was conducted in the animal house of Biology department, Faculty of Science, Soran University; under supervision and approval of local scientific committee and animal care rules. Twenty male albino rats their body weights rang between (250 - 370) gram were used, each five
in one plastic cage with free access for standard rat diet, the house temperature about 25 ± 2 °C and 12/12 hour photoperiod dark and light cycle.

B. Experimental Design

An experiment was designed to investigate that, whether male rats are more sensitive to liver and kidney damage. Animals were divide into two groups, each group contain 10 rats as follow:

Group sham (Not castrated) their scrotum opened and sewed without touching the tests and given lead acetate (80 mg/L D.W) by drinking for 15 days.

Group castrated their testes removed surgically and given lead acetate 80 mg/L D.W by drinking for 15 days.

C. Castration

Rat were anaesthetized by injection a mixture of Ketamine hydrochloride (80 mg/Kg) and Xylazine (12 mg/Kg) intraperitoneally; a small incision (1 – 2 cm) was done on the frontal aspect of scrotum, then testes pulled out gently without bleeding, near abdomen the blood vessels and ducts tightly ligated by absorbable suture (DemTech, England), then after cutting the testes sterilized with Hibitane (5%), and scrotum sewed by Nylon mono filament; all steps were performed for sham group without touching the testes.

D. Blood Sample Collection, Liver and Kidney Organ Weight

Under anaesthetized condition by (Ketamine) (80 mg/Kg)/Xylazine (12 mg/Kg), the blood was taken (8 – 10 mL) by cardiac puncture, and transferred into gel tube (Fl medical, Italy), left standing for 30 minutes, then centrifuged at 2000 rpm/15 minutes, the sera were stored in 3 eppendorf tubes at –20 °C till the assay day. The skin and abdominal muscle were removed, then immediately both kidneys and liver had been cut and weighted.

E. Liver Function Tests

Serum levels of liver enzymes (AST, ALT, and ALP) were measured by automated chemical analyzer (BioTech, Uk).

F. Determination of MDA

Serum concentration MDA was determined according to TBA-MDA method; 0.150 µL added into clean glass test tube, then 1 ml thioarbituric acid (TBA) (0.66%) and trichloroacetic acid (TCA) (17%) were added respectively, after boiling at 95 °C for 45 minutes another 1 mL TCA (70%) would be added; cooled and centrifuged at 2000 rpm for 15 minutes; and the supernatant was read at 532 nm.

G. Statistical Analysis

Statistical Package for Social Science (SPSS) version 16 was used for analysis of data. Results were expressed as Mean ± Standard error. Student t-Test was applied to compare between groups. Figures had been drawn by GraphPad Prism software.

III. RESULTS

This study showed that, giving lead acetate (80 mg/L) through drinking, for fifteen days could decrease significantly (P < 0.05) liver weight of sham group in comparison with castrated rats (Fig. 1).

While, the toxic effect of lead acetate reduced the right kidney weight (3.890 ± 0.077) gram in sham group as compared castrated group, but was none statistically significant (P > 0.05) consideration. The left kidney weight (3.766 ± 0.075) none significantly diminished in sham versus castrated group (3.858 ± 0.099) (Table I).

Furthermore, the serum level of AST was measured by automated chemical analyzer instrument; it showed that, there was significant (P < 0.05) increased in AST as compared to castrated groups (Fig. 2). Whereas, minor in ATL and ALP level, it means that, neither ATL (64.42 ± 10.70) nor ALP (873.8 ± 90.09) concentration in both groups significantly different (Table II).

On the other hand, serum MDA determination would helpful to identified liver injury, but despite harmful effects of lead as a model to liver injury there was not significant (P > 0.05) changes between castrated rats (14.34 ± 0.548) and sham rats (15.08 ± 0.229) (Table II).

### TABLE I

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>(P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KIDNEY WEIGHT OF MALE RAT TREATED BY LEAD ACETATE (80 MG/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right kidney weight (Per 1000 gram)</td>
<td>3.890 ± 0.077</td>
<td>3.977 ± 0.115</td>
</tr>
<tr>
<td>Left kidney weight (Per 1000 gram)</td>
<td>3.766 ± 0.075</td>
<td>3.858 ± 0.099</td>
</tr>
</tbody>
</table>

Sham; Rat’s with intact testes, castrated rat; Rat’s without testes

![Fig. 1. Liver weight of male rat treated by lead acetate (80 mg/L).](image1)

![Fig. 2. Serum AST (IU/L) of male rat as treated by lead acetate.](image2)
TABLE II
SERUM ALT, ALP, AND MDA OF MALE RAT AS TREATED BY LEAD ACETATE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham</th>
<th>Castrated Rat</th>
<th>(P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>64.42 ± 10.70</td>
<td>62.66 ± 4.862</td>
<td>0.786</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>873.8 ± 90.09</td>
<td>752.1 ± 32.00</td>
<td>0.232</td>
</tr>
<tr>
<td>MDA (µ/L)</td>
<td>14.34 ± 0.548</td>
<td>15.08 ± 0.229</td>
<td>0.243</td>
</tr>
</tbody>
</table>

Sham: rat’s with intact testes, castrated rat: rat’s without testes

IV. DISCUSSION

The two experimental animal models have been used to investigate male vulnerability for liver injury. Lead acetate administration through drinking water would produce whole body injury, specifically liver and kidney (Waseem, et al., 2014), also castration is surgical procedure to block androgen hormones, diminishing their concentration inside body fluids such as blood plasma (Duran-Pasten, et al., 2013). The present study tries to explain the role of androgens (Testosterone) in enhancement of many physiological processes in liver and kidney injury, such serum enzymatic activities, and lipid peroxidation.

The weight of liver fallen (P < 0.05) in the sham rats (Fig. 1), while the kidney weight did not decrease significantly according to castrated animals (Table I). Many mechanisms contributes in that phenomenon such as, the testosterone hormone stimulate and enhance liver injury (Hoebedecke, et al., 2013). Much more levels of androgen signaling, reflected by higher testosterone levels may associated with the risen risk of many hepatic disease (Yu, et al., 2000). The induces toxic hepatitis testosterone’s character, also may lead to reduce liver weight and enhances liver cell damage (Timcheh-Hariri, et al., 2012). Beside male sex hormones which has typical effects on liver tissue, administration of high dose of testosterone caused increase kidney tissue damage and kidney weight fallen (Rostami, et al., 2014), but it needs sufficient time, for that reason kidney weight not decrease significantly in sham group, as induces organ injury by lead acetate (Table I).

The activity of AST in castrated rats is significantly (P < 0.05) lower than sham (Fig. 2). Neith ALT nor ALP was decreased significantly (Table I). Consideration of enzyme activity in this investigation, due to testosterone dose dependent risk effects on liver organ, such as enhancing liver fibrosis (Vieira, et al., 2008), “hepatocellular necrosis instead of intrahepatic cholestasis” (Stimac, et al., 2002), androgenic hepatotoxicity includes “genetic cholestatic syndromes” (El Sherrif, et al., 2013), and the varieties physiological action of androgen hormones effects on the body, through wide spread of their receptors on the organ tissues (Coss, et al., 2012).

Furthermore, TBA-MDA showed that there was not significant (P > 0.05) variation between castrated and uncastrated rats (Table II). When, there was obvious relation or interactions between Leydic cell testosterone production and increasing oxidative stress (Chen, et al., 2015). Believed that, this free radical proportional effect on testicular cells and risen lipid peroxidation dependent on time, because the time to produce tissue injury was short, it was 15 days.

V. CONCLUSION

It was clearly demonstrated that, the availability of testosterone would lead to increase liver injury; also the vulnerability of male to liver disease was time dependent, beside that kidney injury was delayed for harmful substance.

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