**Study on Liver Development of Newborn Male Rats form The Mothers Treated with Nortriptyline hydrochloride**

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**INTRODUCTION**

One of the old medicines applicable for long-term treatment of depression is nortriptyline hydrochloride that has been confirmed by FDA on Nov 1964. The medicine is existed in pharmacologic classification of tricyclic antidepressants. The mentioned medicine is in categorization of consumption in pregnancy in D group based on classification of Food and Drug Association [FDA] of America. This is because; some cases of danger for human fetus as a result of using the drum in pregnancy period have been reported and that the drug can enter to mother's milk with low density. In fact, safe consumption of nortriptyline during lactation period has not been confirmed. However, in under specific conditions that the disease is dangerous, according to the doctor, advantages of using the medicine would overcome its disadvantages and the drug would be prescribed [1]. Brands of the drug include Aountyl hydrochloride, Aountyl Alger, nortriptyline, noutylin and pamelour. The drug is soluble in the water and its solution is crystalline. The drug is sensitive to the light and moisture and should be stored in dry place and in dishes resistant against light. Suitable temperature for it is below 30°C for human fetus as a result of using the drum in pregnancy period have been reported an

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**Keywords:** nortriptyline, liver, newborn male rat
The drug would be absorbed immediately after edible prescription and achieves its maximum serum level after 2–4 hours [7]. Mechanism of Action [MOA] of the drug is with controlling reabsorbing pre-synaptic and serotonin inpre-synaptic neurons, which can increase their synaptic density in the central nervous system. It seems that constant use of the drug can cause some changes in level of receptors, which can explain antidepressant effects of the drug to some extent [8]. However, as a result of long-term use of the drug, the body would become resistant against it and also the antidepressants can have undesirable effects on health of cardiovascular system [9]. Metabolism of nortriptyline hydrochloride would be conducted throughdemethylation and hydroxylation and can be appeared in two forms of Cis and trans, which trans form is dominant. In addition, N - dimethyl nortriptyline would be created in high amount. 1-hydroxy nortriptyline Trans is stronger than cis form and acts through being attached to plasma [10]. It includes also hepatic first-pass metabolism. The most common side effects of the drug are as follows: dizziness, headache, palpitations, blurred vision, intraocular pressure, Xerostomia, urinary retention, nausea, constipation and weight gain or loss. However, there are also rare side effects including skin rashes, hives, seizures, and hepatitis. However, appearance of side effects with nortriptyline is less than other tricyclics [11].

Medicines that are currently being prescribed in pregnancy and parturition period for mental diseases, especially in third trimester of pregnancy and close to parturition, can have different effects on the fetus. Therefore, the mother should be absolutely under control of the doctor [12]. Nortriptyline can be transferred through placental barrier and can be then metabolized by the infant [13]. There are some reports, in which some concentrates of the drug have been found in amniotic liquid, which the fetus can be affected by that [14].

Liver is the biggest internal organ, which can provide metabolism, endocrine and exocrine gland actions. The actions include bile production, metabolism of compounds related to diet, detoxification, regulating blood glucose homeostasis through the glycogen storage and controlling blood homeostasis through secretion of blood clotting factors and serum proteins such as albumin [15]. The main place of drug metabolism is in liver. There are also histological observations and also blood tests available for purpose of evaluating liver function, in which measurement of level of some liver enzymes would be evaluated in order to diagnose hepatic inflammations or other failures of liver [16]. According to limitation of conducting experimental studies on human, it seems that no study has been conducted on effects of nortriptyline hydrochloride on liver tissue of infants in the mothers, who have used the drug in their pregnancy period. As the most ant-depressant drugs have many side effects in addition to have medical effect, the present study has attempted to investigate probable side effects of the drug on weight of infants through experimenting liver tissue in newborn male rats.

Methodology:

At the present study, powder of nortriptyline hydrochloride was solved in the distilled water and a homogeneous solution with certain densities was produced for each experimental group. During the experiment, the groups were same in terms of food type, water and environmental conditions and prescription of the drug in pregnancy period was conducted daily in 9-10 am in edible form using feeder syringe.

Categorizing animals and other steps of the experiment:

At the present study, 50 newborn male rats [22-day infants] from Wistar strain were selected and then were weighted and categorized in 5 groups with 10 rats in each group.

Control group: the group included 10 newborn male rats from mothers, which had received no type of solution or drug.

Observation group: the group included 10 newborn male rats from mothers, which had received daily 1cc of the solution [drug] in edible form during their pregnancy period.

Experimental groups of 1, 2 and 3: each group included 10 newborn male rats from mothers, which had received daily 1cc drug solution respectively to 20, 40 and 80mg/kg in edible form.

The 22-day rats in all groups were then weighted and then were anesthetized using ether. Afterwards, their chest was opened and their liver was removed completely. After weighting their liver, it was fixed in the10% formalin in order to provide tissue slides. Then, thin layers were cut out of the liver tissue and were then stained using Hematoxylin and eosin. Then, histological evaluation was performed using microscope.

Statistical analysis methods:

In order to analyze data, SPSS software has been applied. Also, in order to determine effect of the treatment on experimental groups, one-way ANOVA test and following it Tukey Test have been applied in order to test differences among the groups. Relevant diagrams have been also traced using Excel software. In all tissues, p≤0.05 has been considered as significance level statistically.

Results:

After investigation of liver tissue in experimental groups [20, 40 and 80mg/kg], it was observed that hepatocyte cells of liverin minimum experimental group became necrotic comparing to control group in low
level; and in medium experimental group comparing to control group it has become necrosis in higher rate as is illustrated respectively in figures 1-5. In experimental group, the necrosis rate was significantly increased compared to control group than the two previous groups, so that the highest rate of necrosis can be observed in this group. In general, it could be mentioned that necrosis rate of liver tissue can be increased based on drug doze. Weight of rats and weight of their liver was also significantly decreased comparing to control group in p≤0.05 level as it is obvious in table 1.

Table 1: mean value of weight of liver and weight of male rats per gram.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Infant weight [g]</th>
<th>Liver weight [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>112.1±2.47</td>
<td>4.9±0.24</td>
</tr>
<tr>
<td>Observation</td>
<td>10</td>
<td>112.1±3.32</td>
<td>5.6±0.23</td>
</tr>
<tr>
<td>Experimental 1</td>
<td>10</td>
<td>103.6±2.54</td>
<td>4.4±0.28</td>
</tr>
<tr>
<td>Experimental 2</td>
<td>10</td>
<td>79.5±2.55*</td>
<td>3.6±0.08*</td>
</tr>
<tr>
<td>Experimental 3</td>
<td>10</td>
<td>78.1±1.44*</td>
<td>3.5±0.22*</td>
</tr>
</tbody>
</table>

* indicates significant difference with control group [p≤0.05]  
Values have been presented based on mean ± of mean standard error.

Fig. 1: Photo micrograph of liver tissue in control group: ×40 magnification of H and E all hepatocyte cells are healthy.

Fig. 2: Photo micrograph of liver tissue in observation group: ×40 magnification of H & E stained All hepatocyte cells are healthy.

Fig. 3: Photo micrograph of liver tissue in minimum experimental group: H & E stained ×40 Flash is a sign for necrosis of the tissue.

Fig. 4: Photo micrograph of liver tissue in medium experimental group: H & E stained ×40 Flash is a sign for necrosis of the tissue.
The damages can proliferate and body growth and as a result, weight loss would be changed along with the toll by inducing the medicine itself on liver or effect of active metabolite. Nortriptyline is activated metabolite of amitriptyline [22]. Inactivity and failure of liver cells can cause apoptotic pathways or can control some biochemical pathways. Hence, cells would be damaged and finally, this can cause necrosis, cholestasis or bile ducts damages [23].

Medicines can cause hepatic damage [DILI]. Such damage can be created either by toxicity of the medicine itself on liver or effect of active metabolite. Nortriptyline is activated metabolite of amitriptyline [24]. On the other hand, during damaging the liver, IFN-γand TNF-α can cause inflammatory responses and help progress of the toll by inducing the medicine [25]. Active metabolites of medicine may cause inactivity of mitochondria and reduction of energy generation. This function of cell can affect cell death and damage [26]. Inactivity and failure of liver cells can cause immunology actions including adaptive and innate immune responses. Additionally, liver cell damages can be resulted from releasing signals that stimulate activity of other cells such as cooperator cells and natural killer cells [27]. According to studies of Harrison, phenytoin can be changed into active metabolism to cause hepatic damage [28].

Discussion and conclusion:
Comparing results of statistical test indicate that experimental group 2 with receiving 40mg/kg nortriptyline hydrochloride and experimental group 3 with receiving 80mg/kg nortriptyline hydrochloride, comparing to control group, indicate significant decrease in both liver weight and weight of newborn male rats in confidence level of p≤0.05. As it was mentioned before, tricyclic antidepressants with controlling reabsorption of serotonin neurotransmitters and norepinephrine in the terminal of pre-synaptic neurons can increase serotonin and norepinephrine in central nervous system, which can be effective in treatment of depression [17]. Serotonin has a key role in relish and its amount is directly depended on individual’s diet and would be changed along with increase and decrease in tryptophan ratio in the brain [18]. Using protein-rich foods can decrease tryptophan ratio and serotonin in the brain; although eating a low carbohydrate food can have reverse effect. Amount of serotonin in central nervous system is sensitive to tryptophan ratio of nutrition and some satiety factors such as chlristonin [CCK] and Antrostatine in blood circulation. Serotonin receptors can prevent function of Neuropeptide Y, which is a powerful stimulator for hunger and food absorption [19]. Reduction of activity of the neuropeptide can lead to increase in activity of leptin hormone. Leptin can be formed in the body by adipose tissue and would then move to the brain through blood circulation and would affect hypothalamus receptors and cause decrease in relish. Hence, hunger and desire for eating food would be eliminated and then the individual would feel satiety. In fact, serotonin is a part of a network for sending relevant messages of satiety [20]. Probably, the medicine can cause increase in leptin hormone through increasing cerebral serotonin and decreasing function of neuropeptide Y and then it can cause decrease in relish. As a result, reduction of synthesis and protein intake can cause decrease in cell proliferation and body growth and as a result, weight loss. In addition, hepatic gluconeogenesis in newborn rats is imperfect. As a result, density of blood glucose in infant, which has not been feed yet, would drop to 30-40mg/dl and the infant should be relied on stored lipids for purpose of supplying energy before feeding. As a result of the phenomenon, weight loss would be occurred [21].

After investigating the liver tissue, it was found that density of liver tissue in all groups, which received nortriptyline hydrochloride [20, 40 and 80mg/kg], comparing to control group has been significantly decreased. The results indicate that the medicine with the mentioned dozes can cause damage in parenchymal cells of the liver. At the present study, through histological study of provided slides of liver, it was found that hepatic parenchyma cells in control and observation groups are completely normal and with no damage; although in experimental groups under medication, some damages could be observed in hepatocyte cells. The damages can be appeared in form of some pores among cells, which can be increased in groups along with increase in consumed doze of the drug. Hence, probably level of cell damage in liver tissue is in direct relationship with amount of consumed doze. In addition, in regard with estimating mean weight of infants, weight loss has been observed, which is in direct relationship with consumed doze of the medicine too. Tricyclic antidepressant medicines can be metabolized by CYP2D6 enzyme from P450 cytochrome system. Ifa defect is created in this system for any reason, active metabolites of the medicine can cause hepatic damage and necrosis. Hence, the medicine would not be offered to individuals with hepatic failure [22]. On the other hand, medicines can damage liver cells directly. Sometimes, medicines or their metabolites can activate apoptotic pathways or can control some biochemical pathways. Hence, cells would be damaged and finally, this can cause necrosis, cholestasis or bile ducts damages [23].
metabolite through P450 cytochrome system in the liver by epoxide hydrolases. If a defect is appeared in activity of epoxide hydrolase, the active metabolite would be connected to hepatic macromolecules covalently and as a result, this can cause necrosis and hepatic failure [28]. In regard with investigating effect of nortriptyline hydrochloride, it could be mentioned that the medicine can cause hepatic failure induced by medicine and can cause hepatic necrosis through affecting TNF. Moreover, probably similar anti-epileptic medicines may cause liver cell failure during hepatic metabolism processes, since the medicine would be metabolized through P450 cytochromes system. Also, probably the medicine can be considered among toxic actors of liver. According to effects depended on the medicine doze, destruction of hepatic parenchyma and reduction of hepatic cell density can be also a cause for liver to lose weight. However, further studies are required for purpose of gaining final and certain results in this field.

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REFERENCES