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# Evaluation of the effect of methadone on liver toxicity by examining the liver enzymes (ALP, ALT, AST) and Bilirubin total and Bilirubin direct

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## ABSTRACT

Background and Objective: drug abuse and drug dependence are complex multi-dimensional problems. Drug addiction is the second leading cause of death after accidents in males. Injecting drug has raised concerns such as transmission of AIDS and hepatitis. Detoxification or therapeutic interventions alone have not had an effect on reducing drug dependence, such as heroin. Oral methadone is a common international method for treatment of the heroin dependence. By removing or reducing complications of drug leaving, methadone reduces heroin dependence. Methadone is effective for 24 hours and can be used instead of heroin with 3 to 5 times of consumption (a long term effect). Therefore, given what was stated above, this study evaluates the effects of methadone on liver and kidney. Methodology: In this comparative study, two group (one group included 100 people and another group included 50 people) were selected from Rudan prison in Hormozgan province using randomized sampling method and they were evaluated. Their liver toxicity was evaluated by measuring Bilirubin, ALT, AST, and ALP of blood in a human model. Results: The results showed the equality of two groups is not rejected in terms of level of AST and ALT at the significance level of 0.05, and after 6-month and 9-month treatment, no difference was found between them, but with regard to the levels of ALP, bilirubin total, and bilirubin total enzyme, the hypothesis of equality of the enzyme is rejected at the significance level of 0.05 given the test level (0.014). After eliminating the pre-treatment variable, it was revealed that the level of enzymes was significantly higher in 9-month treatment period that that of 6-month treatment period. Conclusion: excessive use of methadone can have an adverse effect on liver enzymes. Thus, to prevent poisoning and death caused by its abuse, it is important to accurately measure the concentration of methadone in the blood or urine of the patients.

**Keywords:** Methadone, liver enzymes, liver toxicity.

## Introduction

Drug dependence and drug abuse in more advanced stages, especially heroin use, are associated with many hazards for people and community and people may not be able to achieve persistent drug avoidance status. Hence, in the case of lack of protective treatment, these patients will have to continue living with supply and use of drugs until the end of their life [1]. As methadone is a strong industrial drug and its addiction is less than morphine and heroin [2] and it has prolonged effect and less euphoria than morphine, it is often used as a drug to control addiction. Methadone is one of the opioids that can affect the normal function of animal and human liver enzymes [3-5]. Methadone binds to the albumin to be carried in blood and it binds to other proteins to be carried in in the lung, kidney, liver and spleen and a balance is gradually created between these tissues and blood in the early days after treatment. After consuming it for several times, it will lead into aggregation. Methadone has direct and indirect effects on the liver, so methadone abuse can cause liver dysfunction [5]. This study was conducted to evaluate the unproven effects of methadone on liver toxicity by measuring liver blood factors including ALP, AST, ALT, Bilirubin total, Bilirubin direct in human model.

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## Methodology

Research population: The population of this comparative research includes all patients receiving methadone treatment for the six and nine months in 2017.

### Sample size and sampling method:

-The sample size of this study included 150 addicted people treated with methadone and selected randomly. In this study, 100 of them were studied in a 6-month treatment period and 50 of them were treated in a 6-month treatment period

-Sampling: At the beginning of the treatment, 5 cc of blood was taken from patients. The tests were performed on the blood of these individuals and the same tests were re-performed for the first group 6 months later. Nine months later, the same tests were re-performed for the second group.

### Blood urea test:

The method used in this study is an enzyme method (Berthollet reaction), in which the ammonia produced by urea hydrolysis by an enzyme urease with a hypochlorite and sodium salicylate yields a green color. The resulting color intensity depends on the value of urea in the sample. The BT3000 device was used for this test. First, the device tests were calibrated by Cal-Centro calibrator, and then, the normal control serum (Centronorm) was given to the device. After reading the control serum by the device, the control result is checked based on blood control brochure. Then, the sample of the individuals is prepared and poured into cup of BT3000 device and it is placed inside the device. The device reads the samples. Using auto-analyzer BT3000 (light absorption by photometric method, the serum concentration of the samples is calculated.

Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase test: The method used for this test is the photometric test method (The International Federation of Clinical Chemistry and Laboratory Medicine or IFCC). After reading the control, a sample of patients is given to the device. This test is read in 341 nm and it is read in 405 nm for alkaline phosphatase and the result is recorded according to the standard.

### Bilirubin total test and bilirubin direct test

The method used in this test is photometric method using 2 and 4 Dichloro-aniline (DCA). The wavelength of 546 nm (540 to 560) should be used in this test. First, the control serum is given to the device. After reading the control, the patient's samples are given to the device and the results are recorded.

## Results

Evaluation of the effect of methadone on liver toxicity with examining the ALP enzyme: The paired test results, presented in Table 1, show that in both of the 6-month and 9-month periods, the mean level of ALP after treatment increased significantly compared to that before the treatment (sig< 0.05).

The normal treatment for ALP (U/L) is between 80 and 306, as shown in Chart 1 with horizontal lines. As seen the mean ALP is in the normal range before and after treatment. However, in individual examining the values, it is seen that 99% of values were in normal range in the 6-month treatment period, which it was reduced to 91% after 6 months of treatment (Table 2). In the 9-month treatment period, 100% of the values were in normal range, which it was reduced to 93.9% after 9 months of treatment (Table 2)

**Table 1: the mean and standard deviation for ALP enzyme and paired t-test results**

| ALP | SEM± mean | | df | t statistic | Test value (sig) |
|---|---|---|---|---|---|
| | Before treatment | After treatment | | | |
| 6 -month | 207.55±4.81 | 215.39±6.37 | 99 | -2.805 | 0.00 |
| 9-month | 215.42±6.17 | 223.49±7.51 | 49 | -2.558 | 0.00 |

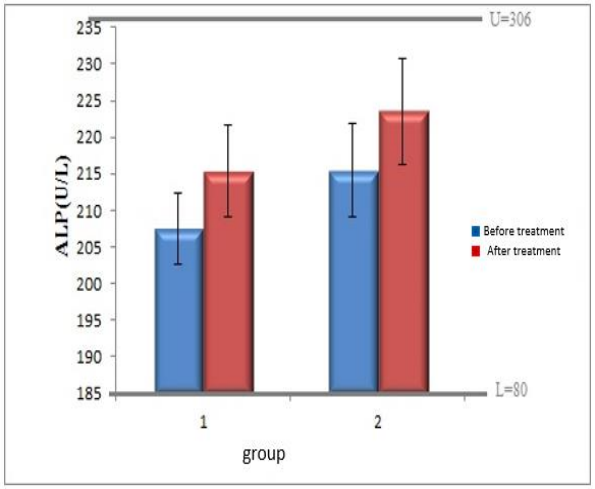


**Chart 1 -** Comparison of the mean ALP with the normal range

### Comparison of methadone effects in the 6-month and 9-month periods by examining the ALP Enzyme:

**Table 2: Results of the covariance analysis-comparison of ALP in 6-month and 9-month treatment groups**

| Source of variations | Sum of squares | df | F test statistic | Test value (sig) |
|---|---|---|---|---|
| Pre-treatment ALP | 445430.9 | 1 | 721.745 | 0.00 |
| Group | 48.38 | 1 | 0.078 | 0.78 |
| Error | 90105.16 | 146 | | |
| Total | 7622258 | 149 | | |

The F value of independent effect (group) was obtained 0.078 and according to the test value (0.78), at a significant level of 0.05, the hypothesis of the equality ALP enzyme level in two

groups was not rejected and no difference is seen in the level of ALP after 6 months of treatment and 9 months of treatment.

## Evaluation of the methadone's effect on liver toxicity by examining the GPT enzyme

The results of paired test, as presented in Table 3, show in both 6-month and 9-month periods, the increase observed in mean GPT after treatment was significant than that of before treatment. The normal range for GPT is less than 41 U/L, shown in Chart 3 with horizontal line. As seen, mean of GPT is within normal range before and after treatment. However, in individual examining the values, it is seen that 98% of the values were in normal range in 6-month period, which they were reduced to 89% after 6 months of treatment (Table 4. In the 9-month period, 100% of the values were in normal range, which it was reduced to 87.8% after 9 months of treatment.

**Table 3: Mean and standard deviation of GPT enzyme and paired t test results**

| GPT | SEM± mean | | df | t statistic | Test value (sig) |
|---|---|---|---|---|---|
| | Before treatment | After treatment | | | |
| 6- month | 22.8±0.82 | 25.75±1.22 | 99 | -2.805 | 0.00 |
| 9 -month | 22.86±1.04 | 26.65±1.52 | 49 | -2.558 | 0.00 |

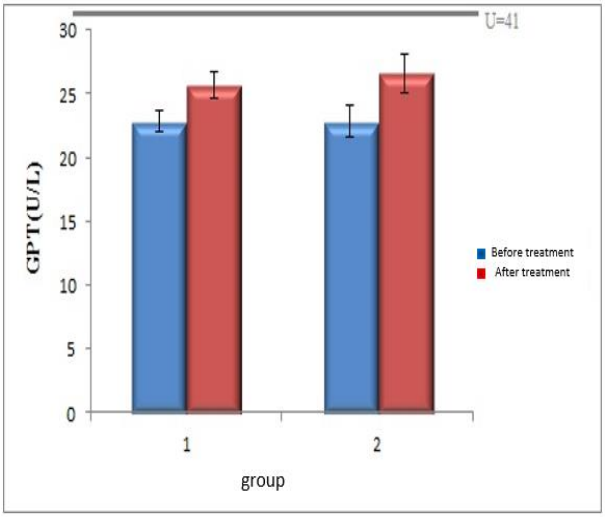


**Chart 2 -** Comparison of the mean GPT with the normal range

## Comparison of the effect of methadone in 6-month and 9-month period by examining the GPT enzyme

According to Table 4, the F value of the interaction of independent variable and the pretest test was 0.83 which is larger than the probability value of 0.01. Therefore, it can be concluded that the null hypothesis (equality of the slope of the regression line) is not rejected, and the assumption of regression slope homogeneity has been observed.

**Table 4: Covariance analysis test for examining the equality of slope of regression**

| Source of variations | Sum of squares | df | F test statistic | Test value (sig) |
|---|---|---|---|---|
| Group* GPT before treatment | 1.647 | 1 | 0.047 | 0.83 |
| Error | 5131.328 | 145 | | |
| Total | 121202 | 149 | | |

After confirming the assumptions of covariance analysis, we test the research hypotheses using covariance analysis.

**Table 5: Results of covariance test - GPT comparison between 6-month and 9-month period groups**

| Source of variations | Sum of squares | df | F test statistic | Test value (sig) |
|---|---|---|---|---|
| Pre-treatment GPT | 15005.37 | 1 | 426.806 | 0.00 |
| Group | 23.24 | 1 | 0.661 | 0.418 |
| Error | 5132.97 | 146 | | |
| Total | 121202 | 149 | | |

According to Table 5, the output of the covariance analysis shows that there is a correlation between the covariate variable and the dependent variable, since the probability of the pre-test was obtained 0.00 and less than 0.05. Thus, null hypothesis on the lack of correlation between the covariate variable and dependent variable is rejected and the effect of pre-treatment variable is significant. The F value of the independent effect (group) was 0.661, and according to the test value (0.418), at a significant level of 0.05, the equality of GPT enzyme in two groups is not rejected, and no difference is seen between 6-month period and 9-month period in terms of GPT enzyme.

## Evaluation of the effect of methadone on liver toxicity by examining the enzyme GOT

The t-test results presented in Table 6 show a significant increase in the mean GOT after treatment in both of the 9-month and 6-month periods compared to that before treatment. The normal range for GOT is less than 37 U/L, which is shown in Chart 3 with horizontal lines. As seen, the mean GOT is between normal range before and after treatment. However, in individual examining of the values, it is observed that in the 6-month period of treatment, 98% of the values were in normal range, and it was reduced to 90% after 6 months of treatment (Table 6). In the 9-month treatment period, 98% of the values were in normal range, and it was reduced to 83.7% after 9 months of treatment.

**Table 6: Mean and standard deviation of mean for GOT enzyme and paired t test results**

| GOT | SEM± mean | df | t statistic | Test value (sig) |
|---|---|---|---|---|

| | Before treatment | After treatment | | | |
|---|---|---|---|---|---|
| 6 -month | 23.3±0.64 | 25.59±0.89 | 99 | -4.05 | 0.00 |
| 9 -month | 23.14±0.86 | 27.53±1.17 | 49 | -5.23 | 0.00 |

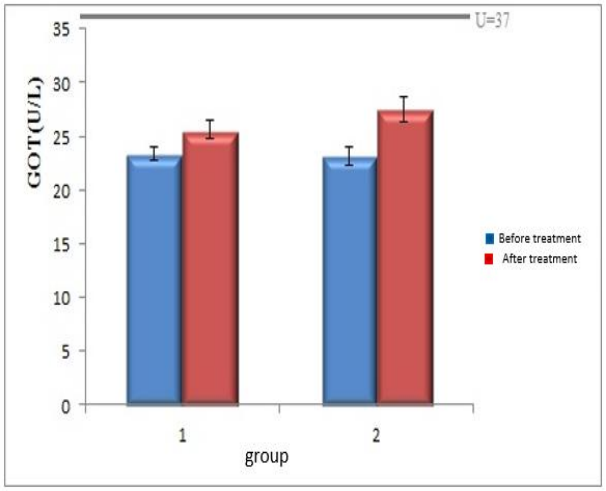


**Chart 3 -** Comparison of the mean GOT with the normal range

## Comparison of the effect of methadone on 6-month and 9-month periods with examining the GOT enzyme

According to Table 7, the F value of the interaction of independent variable and the pretest test is 0.692 and the probability level of the test is 0.407 which is larger than the significant level of 0.01. Therefore, it can be concluded that the null hypothesis (equality of the slope of the regression line) is not rejected, and the assumption of equality of slope of regression has been observed.

**Table 7: Covariance analysis test to examine the equality of slope of regression**

| Source of variations | Sum of squares | df | F test statistic | Test value (sig) |
|---|---|---|---|---|
| Group* pre-treatment GOT | 22.422 | 1 | 0.692 | 0.407 |
| Error | 4696.958 | 145 | | |
| Total | 113766 | 149 | | |

After confirming the assumptions of covariance analysis, we test the research hypotheses using covariance analysis:

**Table 8: Results of covariance test - Comparison of GOT in two groups of 6-month period and 9-month period**

| Source of variations | Sum of squares | df | F test statistic | Test value (sig) |
|---|---|---|---|---|
| Pre-treatment GOT | 6423.014 | 1 | 198.704 | 0.00 |
| Group | 150.301 | 1 | 4.650 | 0.033 |
| Error | 4719.38 | 146 | | |
| Total | 113766 | 149 | | |

According to Table 8, the output of the covariance analysis shows that there is a correlation between the covariate variable and the dependent variable, since the probability of the pre-test was obtained 0.00 and less than 0.05. Thus, null hypothesis on the lack of correlation between the covariate variable and dependent variable is rejected and the effect of pre-treatment variable is significant. The F value of the independent effect (group) was 4.650, and according to the test value (0.033), at a significant level of 0.05, the equality of GPT enzyme in two groups is rejected. After removing the pre-treatment variable effect, the adjusted mean was obtained and it showed that GOT enzyme was significantly higher after the 9-month period treatment than that in 6-month period treatment.

## Evaluation of the effect of methadone on liver toxicity by examining direct bilirubin

The results of the paired t-test, presented in Table 9, show that there is no significant difference in direct bilirubin mean in the 6-month treatment period, but a significant increase was seen in the mean of direct bilirubin after treatment in the 9-month treatment period. The normal range for direct Bilirubin is 0-0.3 mg/dl 1shown in Chart 4 with horizontal lines. As seen, the mean direct bilirubin is in normal ranges before and after treatment. However, in individual examining the values, it is observed that in the 6-month period of treatment, 96% of the values were in normal range, and it increased to 99% after 6 months of treatment (Table 9). In the 9-month treatment period, 100% of the values were in normal range, and it did not exceed the normal range after 9 months of treatment.

**Table 9: Mean and standard deviation of mean for Bilirubin direct and paired t test results**

| Bilirubin direct | SEM± mean | | df | t statistic | Test value (sig) |
|---|---|---|---|---|---|
| | Before treatment | After treatment | | | |
| 6 -month | 0.0909±0.007 | 0.0902±0.006 | 99 | 0.086 | 0.00 |
| 9 -month | 0.0608±0.0026 | 0.0718±0.0044 | 49 | -3.12 | 0.00 |

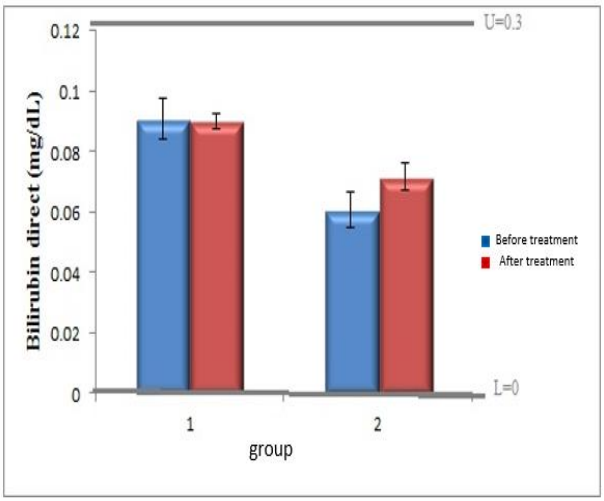


**Chart 4-**Comparison of the mean Bilirubin direct with the normal range

Comparison of the effect of methadone in the 6-month and 9-month period of treatment by examining the bilirubin direct

**Table 10: Analysis of covariance for examining the quality of the slope of regression**

| Test value (sig) | F test statistic | df | Sum of squares | Source of variations |
|---|---|---|---|---|
| 0.03 | 4.788 | 1 | 0.011 | Group* pre-treatment Bilirubin direct |
| | | 145 | 0.343 | Error |
| | | 140 | 1.452 | Total |

After confirming the assumptions of covariance analysis, we test the research hypotheses using covariance analysis:

**Table 11: The results of covariance test - comparison of bilirubin direct between 6-month and 9-month groups**

| Source of variations | Sum of squares | df | F test statistic | Test value (sig) |
|---|---|---|---|---|
| Pre-treatment bilirubin direct | 0.026 | 1 | 10.898 | 0.001 |
| Group | 0.004 | 1 | 1.808 | 0.181 |
| Error | 0.355 | 147 | | |
| Total | 1.452 | 150 | | |

According to Table 11, the output of the covariance analysis shows that there is a correlation between the covariate variable and the dependent variable, since the probability of the pre-test was obtained 0.01 and less than 0.05. Thus, null hypothesis on the lack of correlation between the covariate variable and dependent variable is rejected and the effect of pre-treatment variable is significant. The F value of the independent effect (group) was 1.808, and according to the test value (0.181), at a significant level of 0.05, the equality of bilirubin direct in two groups is not rejected and there is no significant difference between 6-month period treatment and 9-month period treatment in terms of level of bilirubin direct.

## Evaluation of the effect of methadone on liver toxicity by examining bilirubin total

The results of the paired t-test, presented in Table 12, show that there is no significant difference in bilirubin total mean in the 6-month treatment period, but a significant increase was seen in the mean of direct bilirubin after treatment in the 9-month treatment period. The normal range for bilirubin total is 0.1-1.2 mg/dl shown in Chart 5 with horizontal lines. As seen, the mean bilirubin total is in normal ranges before and after treatment. However, in individual examining the values, it is observed that in the 6-month period of treatment, 100% of the values were in normal range, and it decreased to 97% after 6 months of treatment. In the 9-month treatment period, 100% of the values were in normal range, but it decreased to 90% after 9 months of treatment.

**Table 12-Mean and standard deviation of mean for Bilirubin total and paired t test results**

| Bilirubin total | SEM± mean | | df | T statistic | Test value (sig) |
|---|---|---|---|---|---|
| | Before treatment | After treatment | | | |
| 6 -month | 0.555±0.0196 | 0.585±0.0213 | 99 | -0.841 | 0.069 |
| 9 -month | 0.578±0.028 | 0.676±0.033 | 49 | -3.125 | 0.003 |

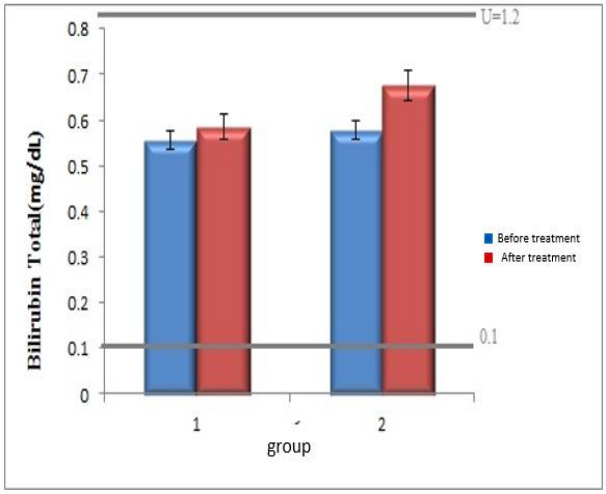


**Chart 5-** Comparison of the mean Bilirubin total with the normal range

Comparison of the effect of methadone in the 6-month and 9-month period of treatment with examining bilirubin total

**Table 13: Analysis of covariance test for examining the quality of the slope of regression**

| Source of variations | Sum of squares | df | F test statistic | Test value (sig) |
|---|---|---|---|---|
| Group* pre-treatment bilirubin total | 0.042 | 1 | 1.392 | 0.24 |
| Error | 4.413 | 146 | | |
| Total | 64.21 | 150 | | |

After confirming the assumptions of covariance analysis, we test the research hypotheses using covariance analysis:

**Table 14: The results of covariance test - comparison of bilirubin total between 6-month and 9-month groups**

| Source of variations | Sum of squares | df | F test statistic | Test value (sig) |
|---|---|---|---|---|
| Pre-treatment bilirubin total | 2.684 | 1 | 88.545 | 0.0 |
| Group | 0.189 | 1 | 6.22 | 0.014 |
| Error | 4.455 | 147 | | |
| Total | 64.210 | 150 | | |

According to Table 14, the output of the covariance analysis shows that there is a correlation between the covariate variable and the dependent variable, since the probability of the pre-test was obtained 0.00 and less than 0.05. Thus, null hypothesis on the lack of correlation between the covariate variable and dependent variable is rejected and the effect of pre-treatment variable is significant. The F value of the independent effect (group) was 6.22, and according to the test value (0.014), at a significant level of 0.05, the equality of bilirubin total in two time periods is rejected and the level of bilirubin total is significantly higher in 9-month period than that in 6-month period.

## Discussion and Conclusion

To compare the effect of methadone in 6-month and 9-month periods, by examining the ALP enzyme at a significant level of 0.05, the hypothesis of the ALP equality in two groups is not rejected and there is no difference between 6-month and 9-month period treatment in terms of level of ALP. The results of this study are in line with those of the research conducted by Lipton et al in 1983, which examined the detoxification of heroin-addicted people and did not observe adverse effect of methadone on the liver [6]. Based on the results, the hypothesis of equality of GPT enzyme in two groups is not rejected and there is no difference between 6-month and 9-month period treatment in terms of level of GPT. In this regard, Wu et al. (1993) evaluated the inhibitory effect of methadone on cytochrome (CYP2D6) P450 2D6 and found that methadone inhibited microsome (CYP2D6), and accordingly, it disturbs liver function [7], which this result has little difference with the result of this study. The results indicate that the hypothesis of equality of GOT enzyme in two time periods is rejected and the GOT enzyme level is significantly higher in 9-month period than that of 6-month period of treatment. In a study conducted in New York in 1976, patients were treated with high doses of methadone after taking alcohol and a high dose of barbiturate [8]. In 7% of the cases, alkaline phosphatase (ALP) increased [9], which is in line with the results of this study. The results show that there is no significant difference in the mean of bilirubin direct in the 6-month period of treatment, but in the 9-month period of treatment, a significant increase was seen in the mean of bilirubin direct after treatment compared to before treatment. In addition, at the significance level of 0.05, the bilirubin direct equality in to groups is not rejected and there is no difference in bilirubin direct levels after 6 months and 9 months of treatment. Given the test value (0.014), at a significant level of 0.05, the hypothesis of equality of bilirubin total in two periods of time is rejected. After removing the effect pre-treatment variable, bilirubin total level was significantly higher in 9-month period of treatment than that of 6-month period of treatment. Ali Nejad et al in 2016 investigated the effect of methadone on the kidney and found that methadone causes many abnormalities, including rhabdomyolysis [10], which is consistent with the results obtained on the level of total bilirubin.

## References


1. Ministry of Health and Medical Education. Opioid agonist drugs treatment protocol. 2nd ed. Tehran, Iran: Ministry of Health and Medical. Education; 2005. [In Persian]
2. Garrido, M. a. J. and I. F. Trocóniz. 2. Methadone: a review of its pharmacokinetic/pharmacodynamic properties. J. Pharmacol. Toxicol. Methods.1999, 66-61 :(2)42
3. Kmieć Z. "Cooperation of liver cells in health and disease". Adv Anat Embryol Cell Biol. 2001;161: III–XIII, 1–151. PMID 11729749.
4. Eslami Shahrbabaki M, Haghdoost AA, Mashaiekhi A, Khalili N, Amini Ranjbar Z, Ghayomi A. Effects of Methadone on Liver Enzymes in Patients Undergoing Methadone Maintenance Treatment. Addiction Health J. 2012; 4 (3-4): 111- 115.
5. Kreek MJ. Medical Safety and Side Effects of Methadone in Tolerant Individuals. Am Med Association J. 1973; 223 (6): 665- 668.
6. Lipton DS, Maranda MJ. Detoxification from heroin dependency: an overview of method and effectiveness. Adv Alcohol Substance Abuse.1983; 2: 31-55.
7. Wu D, Otton S, Sproule B. Inhibition of humancytochrome P450 2D6 (CYP2D6) by methadone, British journal of clinical pharmacology, 35 (1993) 30-34.
8. Persky VW, Goldfrank LR. Methadone overdoses in a New York City hospital. JACEP 1976; 5(2): 111-3.
9. Liu S, Wang R, Increased analgesia and alterations in distribution and metabolism of methadone

bydesipramine in the rat, TheJournal of pharmacology andexperimental therapeutics, 195 (1975) 94-104.

10. Alinejad S, Ghaemi K, Abdollahi, Mehrpour O. Nephrotoxicity of methadone. Medical Toxicology and Drug Abuse Research Center (MTDRC); SpringerPlus (2016) 5:2087.