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Morphine Sulphate Toxicity on Liver Function Tests in Fructose-Induced Insulin Resistant Male Rats

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Abstract

***Background*:** Since liver is a gland which has an important role in drug metabolism, the present study was conducted to evaluate the effect of a single dose and repeated administration of morphine on LFT, blood sugar and fasting insulin resistance index in fructose- fed male rats.

***Materials and Methods*:** The experiment was performed on 36 Wistar-Albino male rats, which were divided into a control (A) and three tests groups (B, C and D). The control group consumed tap water, but the test groups consumed fructose-enriched water (10%, w/v) and received null, single, and repeated doses of morphine, respectively. At the end, animals were anesthetized and blood samples were collected. Liver enzymes, insulin and insulin resistance were measured. Data were analyzed by SPSS-11, using ANOVA and Tukey tests as post hoc test. Results were expressed as mean±SD and Statistical differences were recognized significant by $p<0.05$.

***Results*:** The results showed that all test groups were insulin resistant; alanine aminotransferase (ALT) and asparatate aminotransferase (AST) activity values in group D significantly increased compared to other groups while its plasma glucose and insulin values showed a significant decrease in comparison to other test groups.

***Conclusion*:** It seems that repeated morphine administration can affect liver function test (LFT) and fasting Insulin resistance index (FIRI) in fructose- fed male rats.

Introduction

Diabetes mellitus is a major endocrine disorder and is a growing health problem in most countries [1]. It afflicts a large number of problems in social condition throughout the world [1- 3]. Diabetes mellitus is clinically recognized by chronic elevation of the glucose level in the blood and is often accompanied by symptoms of, polyuria, polydipsia, polyphsia, hyperlipidemia and weight loss [4]. The prevalence of diabetes is, unfortunately, increasing in most populations, being more common in developing countries [5, 6]. Insulin resistance is a major metabolic disorder that has an important role in inducing the diabetes mellitus and hypertension [7, 8]. In addition, investigations have shown that morphine (MOR) and its metabolites induce hypoglycemia [9-11] and improve the glucose tolerance in diabetic patients [12, 13]. Moreover, the influence of acute morphine intoxication on glycolytic enzymes in liver and its toxicity has already been reported [14-19]. In Animals after administration of morphine, the serum glucose was increased but the blood insulin lessens than control group [20]. The experimental research showed that enriched fructose- food could not cause liver toxicity in rats [21, 22] although another study indicated that it can produce dyslipidemia, insulin resistance and some aspects of the metabolic syndrome [23].

To our best knowledge, since only a few diabetic patients seem to consume opiate agents and also liver has a central role in drug metabolism, the present study was carried out to elucidate the effect of a single dose and repeated administration of morphine sulphate on liver function test (LFT), blood sugar, insulin and fasting Insulin resistance index (FIRI) in fructose- induced insulin resistant male rats.

Materials and Methods

The experiment was performed on 36 adult Wistar-Albino male rats, weighing 200±220 gr which were separately housed in cages (one rat per cage). Animals had free access to water and food pellet and were kept in a standard condition: room with temperature of 23±2ºC and 12 h of fixed artificial light period (Timer Model: SUL180a, AC220V. China, 6 Am to 6 Pm). Insulin resistance was induced by fructose solution 10% (w/v) in tap water for 60 days. All rats were weighed by GOTTL KERN & SHON (first weight) and divided into sham

control (A) and three tests groups randomly (B, C and D, N=9): Sham control group (A**)** consumed only tap water while test groups (B, C, D) were provided with fructose-enriched water (10%, w/v).

(I)- First group (B) consumed water of fructose-enriched (10%) for 60 days to produce insulin resistance [22]. (II)-second group (C) received a subcutaneous single dose of morphine sulphate (10 mg/kg) 30 min before blood samples were collected. (III)-third group (D) were given fructose-enriched water and, on the basis of previous studies, were addicted by subcutaneous injection of morphine sulphate twice a day(8 am and 4 pm) for 4 days with increasing doses: 10, 20, 30, 40 mg/kg /injection [24]. Morphine sulphate administration in group D continued throughout the treatment with a smaller dose for a month.

All experimental and control rats were weighed (final weight) after morphine administration. At the end of the experiment, all animals were anesthetized with Ketamin (Specia Hungry) and sacrificed. Then blood samples were collected from their cervical vein. The collected blood samples were centrifuged at 3000 r.p.m. for 20 min. Following that, serum specimens of each animal were divided into two parts in clean small tubes, the first part of which was kept at 4-8ºC for measuring liver enzymes and glucose level within 24 hr. The second part was quickly frozen at -20ºC for insulin measurement.

Liver enzymes were measured via ordinary methods. Fasting Blood Glucose (FBS) was measured by Glucose oxidase-peroxidase methods and insulin was measured by ELISA technique (kits ultrasensetive Rat Insulin ELISA Company, DRG Instrument GmbH, Germany Frauenbergstr. 18, and D-35039Marburg). Fasting Insulin Resistance index was measured via HOMA test (fasting Insulin resistance index = fasting glucose (mg/dl) ×fasting insulin (iu/l) × 25/100 [25]. The design of the study was approved by the Ethic Committee of Zahedan University of Medical Sciences and Health Services, Zahedan, Iran (1731, 28-6-2005).

Results were reported as mean±SD. To confirm the normal distribution, the data were analyzed by one-samples Kolmogrov-smirnov test and then by Levine's test for multi comparison were employed to compare differences among experimental group. Statistical difference was recognized significant by $p<0.05$. All statistical analysis were performed using SPSS-11 for windows software system

Results

Results showed that enriched-fructose water produced insulin resistance in all test groups. Fasting insulin resistance index values in A, B, C and D groups were 0.35, 1.4, 1.82 and 0.89, respectively.

Moreover, the pretest FBS was similar in all groups, and the posttest FBS concentration value increased in all test groups given water supplementation with 10% (w/v) of fructose (Table 1). In group D, FBS and insulin values decreased compared to those in other test groups (Table 1).

Initial body weight was similar in all four groups but final weight significantly decreased in group D compared to that of group B (Table 2). Liver enzymes (AST and ALT) activity values increased in group D compared to those of all other groups, however, these values did not show a significant difference among other three groups (Table 2).

Table 1. Comparison of FBS, insulin and water intake in studied groups after morphine administration.

Groups	Pretest FBS (mg/dl)	Post test FBS (mg/dl)	Insulin (IU/l)	Water intak (ml)
A	81.62±14.6	72.87±17.77	0.11 ±0.04	34±11.92
B	79.2±15.33	126.8±34.75	0.25 ±0.17	50.68±12.62
C	83.4±10.75	152.2±26.48	0.27 ±0.13	53.17±13.06
D	93.1±14.21	118.2±17.81 *	0.16 ±0.09+	69.07±9.08 ++

Numbers represent mean ± SD, N=10, *,+, ++=$p<0.05$
*compared with group C and group B.
+compared with group C and group B
++compared with group B and group C

Table 2. Comparison of ALT, AST (IU/L), first and final weight (gr) in studied groups after morphine morphine administration.

Groups	ALT (IU /l)	AST (IU /l)	First weight (gr)	Final weight (gr)
A	207±20.74	170.7±10.08	197±17.5	261.37 ±29.24
B	183.1±14.19	173.4±15.21	192.7±29.8	256.9±27.7
C	217.87±21.44	181.25±11.62	196.8±23.4	258.3±19.24
D	273±21.72 *	213±12.2 +	198.6±29.1	225.4±21.4 ++

Numbers represent mean ± SD, N=10, *, +, ++=$p<0.05$
*compared with group A.
+compared with group A
++compared with group B

Discussion

Results showed that enriched-fructose water produced insulin resistance in all test groups. Fasting insulin resistance index values, based on HOMA test in A, B, C and D groups, were 0.35, 1.4, 1.82 and 0.89 respectively.

Our results are also in accordance with those of Lee et al. in 2009 who showed that dietary fructose consumption causes obesity, insulin resistance and dyslipidemia in laboratory animals [23] and demonstrated that feeding rats with a rich diet in fructose does not affect hepatic expression of inflammatory pathways [23].

The present results, moreover, indicated that the AST and ALT activity values in the addicted group (D) were higher than those of control and other test groups (Table 2). This section of the results is similar to that of Borzelleca et al. who reported increased levels of aminotransferase (ALT) and aspartate aminotransferase (AST) in rats after long-term usage of morphine [26].

Nogamatsu et al. in 1986 demonstrated that addition of morphine components to the artificial culture of isolated rat hepatocytes induced a marked decrease in the cells and resulted in cell death [25].

In addition, these results are in agreement with those of Miskevich et al. whose report suggested that chronic morphine administration causes marked inhibition of the peroxide-utilizing antioxidants and produces condition for liver toxicity [18]. Our conclusion in group D as the same as Sumathi et al., which investigated that morphine hydrochloride geneses intoxicated in rats which received 10-160 mg/kg body weight of intraperitoneally for 21 days [26].

Unlike Johansen et al. in 1992 who demonstrated that intravenous administration of morphine causes increased blood glucose in the rats [20], our results indicated that in the addicted group of D, FBS value decreased compared to that of other test groups (Table 1). The differences of our results from those of Johansen et al. are probably due to the difference in the employed methods and combination of morphine.

Molina et al. showed that morphine and morphine 6 glucoronid administration causes an increase in blood glucose, epinephrine, nor epinephrine, corticosteroids and glucagon [15]. Our results, however, showed that insulin value in group D increased compared to other test groups (Table 1) that may be due to decreasing blood sugar in this group. A major limitation in our study was that we could not measure catecholamine, corticosteroids and glucagon.

In group D, the morphine dependent, decreasing fasting blood sugar might be due to the idea that increased morphine sulphate may activate both neuronal and hormonal reflexes to stimulated glycolysis and increased glucose uptake by lipid and skeletal muscles tissues so as to maintain homeostasis of blood glucose [15].

Jairaj et al. reported that codeine, morphine, and oxycodone administration in incubations rat cell culture cause poisoning in hepatocytes by decreasing total protein content and depletion lactate dehydrogenase in test cells compared with those of control group [28]. Our findings in the present study revealed that serum AST and ALT enzymes activity in group D were increased compared with those of the other groups. This section of our findings was accorded with those of Jairaj et al. [28].

In addition, the present study revealed that in morphine dependent rats, water intake significantly increased in fructose- fed male rats, compared to sham control that can be due to the effects of morphine sulphate on water regulating center in the central nervous system which induces water intake.

The present study indicated that long term use of morphine can affect LFT and FIRI in fructose-fed male rats. Future studies are recommended to investigate the exact mechanism of morphine on liver toxicity in fructose- induced insulin resistance in male rats.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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