

Effects of Opium Addiction on Some biochemical parameters on Diabetic Rats

ABSTRACT

These days, opium consumption has increased among people. Opium latex contains approximately 12% of the analgesic alkaloid morphine, which is processed chemically to produce heroin and other synthetic opioids for medicinal use and for the illegal drug trade. This study was carried out to study the effect of opium on biochemical parameter and histopathological changes of kidney and liver in experimental diabetic rats animals, due to opium abuse. Twenty four albino rats were divided into three groups and traditional opium given orally (10mg / kg B w) to all experimental rats except the control negative group, for 90 days. Diabetes mellitus was induced in adult male albino rats, using intra-peritoneal injection of 120 mg/kg BW. Blood glucose, Serum insulin, Total protein, Urea, Creatinine, Alanine aminotrasferase (ALT), Aspartate aminotransferase (AST), Triglycerides (TGs), Total cholesterol, were measured. and histological examination will done(delete). The data showed that, there was a decrease in levels of serum total protein, ALT, AST cholesterol and triglycerides in diabetic addicted animals compared to non-addicted diabetic animals, while creatinine and urea in addicted diabetic animals were higher. The results showed that, opium increase serum insulin and decreases serum glucose but non not significant, and thus adds this due to metabolic disorders in diabetic animals. These results suggest proved that opium reduces blood glucose in diabetic animals, and but mechanism of this effect is unclear.

Key words: Opium, alloxan, biochemistry parameters and rats

INTRODUCTION

Opium (poppy tears, *lachryma papaveris*) is the dried latex obtained from the opium poppy (*Papaver*

somniferum). Opium latex contains approximately 12% of the analgesic alkaloid morphine, which is processed chemically to produce heroin and other synthetic opioids for medicinal use and for the illegal drug trade. The latex also contains the closely related opiates codeine and thebaine and non-nalgesic alkaloids such as papaverine and noscapine. Opium is obtained by drying or partly drying the latex obtained by incision from the unripe capsules of *Papaver somniferum*. It has a strong characteristics odour and a bitter taste, and contains about twenty five alkaloids including morphine (4-17 %), noscapine (2-9%), codeine (0.3-4%), in addition to smaller proportions of thebaine, narceine, papaverine and hydrocotarnine^(1,2). Opioids are used primarily for analgesia. In addition they are also used as cough suppressants and for the treatment of diarrhea. Occasionally, opioids are also used for sedation before surgery and as supplement to anesthesia³. Regarding the effect of opium on diabetes disease, there are controversies from the reduction in blood glucose factor^(4, 5). It is known that opium smoking could lead to serum glucose and high density lipoprotein alterations and thus worsens metabolic disorders in diabetics⁶. In addition, opium plays a pivotal role as a risk factor for coronary artery disease especially in diabetics and the amount of opium consumed was significantly associated with the severity of coronary atherosclerosis in these patients⁷. Opium is generally absorbed after oral administration and undergo hepatic metabolism, and rapidly cleared from blood and stored in kidney, liver, brain, lung, spleen, skeletal muscle, and placental tissue. Most of the opium

metabolites are excreted through the kidneys⁸. Chemical mechanisms of the selective action of alloxan ~~was(delete)~~ inhibits the glucokinase enzyme originally extracted from the liver and present in pancreatic β -cell. Glucokinase is the part of the signal mechanism by blood glucose levels monitor insulin production. A second important theory of the specific toxic action of alloxan was the reaction with-SH (thiol) groups which liberate peroxides, superoxides and hydroxyl radicals, all highly cytotoxic⁹. It was reported that opium used for the treatment of alloxanoised rats, which, restores some of the normal functions of β -cell in secreting insulin. Normal function of pancreatic β -cell was assessed by bringing back nearly normal blood glucose and normal insulin level, suggesting normal β -cell response to glucose⁽¹⁰⁻¹²⁾.

MATERIALS AND METHODS

1- Chemicals, materials and Kits

Alloxan tetrahydrate was purchased from Sigma Company, USA. Glucose kits were purchased from Böehringer Germany. Urea, Creatinine, ALT, AST were purchased From Bio Merieux, France. Other kits were purchased from Sörin Biomedica, Italy. Crude opium was obtained from a case in our lab Ministry of Justice, Egypt, under lenience of Ministry of Justice, Egypt.

2- Animals

Albino Wistar male rats at the age of 15 weeks, weighting 220-250g were obtained from the animal house faculty of Pharmacy, University of Assiut- Assiut, Egypt.

3- Preparation of alloxan-induced Diabetes

Alloxan was dissolved in sterile distilled water. Diabetes was induced in 16 rats by intra-peritoneal injection of 120 mg / kg. The animals were fasted 12 h before ~~and after the~~ alloxan injection. The animals with blood glucose above 250 mg/dl, as well as with polydipsia, polyurea and polyphagia, which last for at least one week, were selected for the experiments.

4- Experimental procedure

The experimental procedure was ~~conducted~~ carried out in accordance with the guide ~~of~~ on the care and use of laboratory animals approved by the committee of Assuit University. It was ~~conducted~~ carried out in the period from January 2015 to August 2015. The animals were housed in cages provided with rice husk as ~~bedding~~ building materials and kept under ambient temperature of $23 \pm 2^{\circ}\text{C}$, fed on a standard pellet diet and water *ad libitum*, and kept in the laboratory condition for 1 week to adapt the climatic condition and for the commencement of treatment protocol. The animals divided into three groups, each group contained (8) animals. The first group was diabetes, the second group, diabetes animals ~~which~~ given opium (10mg /kg BW) for 90 days, while the third group was normal health. The animals fed with ordinary pellet food. Two milliliter of blood samples was collected in heparinized containers for biochemical tests. Plasma was separated by centrifugation at 3000 rpm for 5 min. Total proteins, glucose, cholesterol, Triglycerides, enzyme kidney (urea and creatinine), and the enzyme activities of (GPT, and ALP) were measured

spectrophotometric by using commercial kits. Blood glucose was determined according to the method described by Trinder¹³. The blood glucose levels¹⁴ and Insulin¹¹ were measured at the end of 3, 7, 10, 15, 20, 25 and 30 days, while Insulin determined by means of an enzyme-linked immunosorbent assay (ELISA) kit (Ultrasensitive Rat Insulin ELISA from Mercodia). at the Middle Eastern Region Radioisotope center for the Arab countries in Cairo. Total protein was determined by Doumas¹², Liver functions (Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined by Reitman and Frankel¹⁵, kidney functions (Urea by Patton¹⁶ and Creatinine determined by Henry¹⁷), while Cholesterol and Triglycerides were determined by Lukenes¹⁸.

STATISTICAL ANALYSIS

The results are expressed as mean $\pm$ SD. The significance of the differences in the values was performed by one – way ANOVA test and Duncan's multiple range tests. $P < 0.05$ was considered to be significant difference.

RESULTS AND DISCUSSION

(1) Diabetic control rats

The intra-peritoneal injection of alloxan in a dose 120 mg/kg body weight was a convenient dose for the induction of diabetes in rats. Blood glucose and insulin level were determined as shown in table (1) and other biochemical parameters were shown in table (2).

Diabetes, showed values of glucose levels table (1). After 3 days (253±7 mg/dl), after 7 days (338±6), after 10 days (442±5), while after 15 days all animals were dead. Diabetes, showed very low values of insulin levels as shown in table (1). After 3 days (6.2±2 µu/ml), 7 days (2.9±3 µu/ml), 10 days (1.6±2 µu/ml), while 15 days all animals were dead.

Table (1) Blood glucose and insulin levels of normal and diabetic rats.

Time	Normal rats		Diabetic Rats	
	Glucose (mg/dl)	Insulin (µu /ml)	Glucose (mg/dl)	Insulin (µu /ml)
3 days	98 ± 3	16.6 ± 2	253 ± 7	6.2 ± 2
7 days	94 ± 2	16.9 ± 6	338 ± 6	2.9 ± 3
10 days	95 ± 4	16.9 ± 5	442 ± 5	1.6 ± 2
15 days	100 ± 2	16.4 ± 3	Dead	Dead
20 days	99 ± 6	16.5 ± 2	Dead	Dead
25 days	96 ± 5	16.8 ± 4	Dead	Dead
30 days	97 ± 3	16.5 ± 2	Dead	Dead

The obtained results showed that, a significant increase in the level of plasma protein concentration of diabetes (12.3±0.8mg/dl) compared to control group (7.5±0.27mg/dl), p-value<0.05 (0.033), while there was a significant increase in the mean value of plasma urea Level of the control diabetes (42±3 mg/dl) compared to that of the normal control group (16.9±2.1 mg/dl) p-value<0.05 (0.029).

The results showed that, there was a significant increase in of parameters, creatinine level (4.2±0.32 mg/dl), ALT (78±5 U/l), AST (71±6 U/l), Triglycerides (233±4 mg/dl), and cholesterol (85±2 mg/dl) in diabetes compared with normal control animals (0.6±0.2 mg/dl) p-value<0.05 (0.019), (35±10 U/l) p-value<0.05 (0.039), (42±8 U/l) p-value<0.05 (0.049), and (116±6 mg/dl) p-value<0.05 (0.045), (52±5 mg/dl) p-value<0.05 (0.042), respectively. Table (2).

Table (2): Plasma concentration of total proteins, Urea, Creatinine, ALT, AST, TGs and Cholesterol for the normal and diabetic rats.

	Total proteins (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	ALT (U/l)	AST (U/l)	TGs (mg/dl)	Total cholesterol (mg/dl)
Normal rats	7.5 ± 2.7	16.9± 2.1	0.6 ± 0.2	35±10	42 ± 8	116 ± 6	52 ± 5
Diabetic rats	12.3± 0.8*	42.0 ± 3*	4.2 ± 0.32*	78 ± 5*	71 ± 6*	233± 4*	85 ± 2*

P value <0.05

(2)Treatment of diabetic rats with opium.

When, alloxanoised animals treated with opium, the results showed that, there was a decrease in blood glucose level, this agreement with Azod¹⁹, which demonstrated that opium decreases FBS (fasting blood sugar) temporarily in diabetic patients but it had no clear and long-lasting effects on blood glucose and HbA1c, and increase in insulin levels but not significant compared with diabetic rats, as follows: after 3 days (218±6 mg/dl), 7 days (210±7 mg/dl) 10 days (186±3 mg/dl), 15 days (183±4 mg/dl), 20 days (179±4 mg/dl), 25 days (165±3 mg/dl), 30 days (128±5 mg/dl) as shown in table (3). But in case of insulin levels, the results as follows: after 3 days (7.6 ±5 µu /ml), 7 days (7.9±3 µu /ml), 10 days (10.6±2 µu /ml), 15 days (12.8 ±6 µu /ml), 20 days (10.8 ±4 µu /ml), 25 days (11.4±8 µu /ml), 30 days (13.2±2 µu /ml). Table (3)

Table (3): Blood glucose and insulin **level** effects after treatment of alloxanoised rats with opium.

Time	Normal rats		Diabetic Rat		Addicted Diabetic rats	
	Glucose (mg/dl)	Insulin (μ u /ml)	Glucose (mg/dl)	Insulin (μ u /ml)	Glucose (mg/dl)	Insulin (μ u /ml)
3 days	98 $\pm$ 3	16.6 $\pm$ 2	253 $\pm$ 7	6.2 $\pm$ 2	218 $\pm$ 6	7.6 $\pm$ 5
7 days	94 $\pm$ 2	16.9 $\pm$ 6	338 $\pm$ 6	2.9 $\pm$ 3	210 $\pm$ 7	7.9 $\pm$ 3
10 days	95 $\pm$ 4	16.9 $\pm$ 5	442 $\pm$ 5	1.6 $\pm$ 2	186 $\pm$ 3	10.6 $\pm$ 2
15 days	100 $\pm$ 2	16.4 $\pm$ 3	Dead	Dead	138 $\pm$ 4	12.8 $\pm$ 6
20 days	99 $\pm$ 6	16.5 $\pm$ 2	Dead	Dead	179 $\pm$ 4	10.8 $\pm$ 4
25 days	96 $\pm$ 5	16.8 $\pm$ 4	Dead	Dead	165 $\pm$ 3	11.4 $\pm$ 8
30 days	97 $\pm$ 3	16.5 $\pm$ 2	Dead	Dead	128 $\pm$ 5	13.2 $\pm$ 2

Alloxanoised animals treated with opium **showed** lower values of serum total protein (9.3 $\pm$ 02 mg/dl) **p-value<0.05 (0.042)**, which agree with Mehdi Mahmoodi²⁰, who found that addicted diabetic males showed lower levels of total protein **comparable** to non-addicted diabetic **patients animals**. The results showed that, there was a decrease level of cholesterol and triglycerides, (75 $\pm$ 4 mg/dl) and (199 $\pm$ 6 mg/dl) **p-value<0.05 (0.039)**, **p-value<0.05 (0.035)** respectively; which **not did't agree** with Azod¹⁹, who demonstrated that, it had no clear and long-lasting effects on the level of cholesterol and triglycerides compared to non-addicted diabetic animals, but in case of cholesterol, **there was** agreement with Mahmoodi²⁰, who found that there was a lower level of total cholesterol compare to non-addicted diabetic **animals patients Mahmoodi²⁰**.

Results clear that, there was a decrease level of AST and ALT, (48 $\pm$ 3 U/l), (44 $\pm$ 7U/l) **p-value<0.05 (0.045)**, **p-value<0.05 (0.038)** respectively, which **didn't** agreement with Kharchenko²¹, who demonstrated, opium addiction caused liver damages, and increased

the activity of AST, ALT, but this agreement with Kharchenko²¹ in case of ALT, compare to non-addicted diabetic patients animals.

Creatinine and urea in addicted diabetic animals were higher (6.5 ± 0.25 mg/dl) and (73 ± 0.43 mg/dl) $p\text{-value} < 0.05$ (0.045) $p\text{-value} < 0.05$ (0.035), respectively compared to non-addicted diabetic animals, which didn't agreement with our findings Helmstädter²², who reported that, no changes were found in, blood urea, blood creatinine levels among; opium addicts, and healthy control, but uric acid level was significantly lower in opium addicts compared to healthy control.

Table (4)

Table (4): Concentration of total proteins, Urea, Creatinine, ALT, AST, TGS and cholesterol after treatment of alloxanoised rats with opium.

	Total proteins (mg/dl)	Urea (mg/dl)	Creatinine (gm/dl)	ALT (U/l)	AST (U/l)	TGS (mg/dl)	Total cholesterol (mg/dl)
Normal rats	7.5 ± 0.27	16.9 ± 2.1	0.6 ± 0.2	35 ± 10	42 ± 8	116 ± 6	52 ± 5
Diabetic rats	$12.3 \pm 0.8^*$	$42 \pm 3^*$	$4.2 \pm 0.32^*$	$78 \pm 5^*$	$71 \pm 6^*$	233 ± 4	85 ± 2
Addicted Diabetic rats	$9.3 \pm 0.2^*$	$73 \pm 0.43^*$	$6.5 \pm 0.25^*$	$44 \pm 7^*$	$48 \pm 3^*$	$199 \pm 6^*$	$75 \pm 4^*$

Although 63 years passed since the discovery of experimental models for the induction of diabetes by injection of alloxan and new β -cell lines are established. Programs of treatment of diabetes mellitus didn't show any remarkable advance. According to the results of our present study, it was found that opium caused a decrease in blood glucose and non-significant increase in insulin levels. The mechanism of this effect is unclear, and showed the other effects of opium addiction on some organs function such as its bad effects on kidney functions liver

functions on diabetes mellitus in addition to other disorders as a result of that important disease. In conclusion according to the obtained results of this study we suggest that opium consumption in diabetic patient isn't useful in spite of public opinion and some of the previous findings.

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