Original Research Article

Effects of Opium Addiction on Some biochemical parameters in 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 opiates for medicinal use and for the illegal drug trade. This study was carried out to study the effect of opium on biochemical parameter changes of kidney and liver in experimental diabetic 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. 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 not significant, this due to metabolic disorders in diabetic animals. These results proved that opium reduces blood glucose in diabetic animals, but the mechanism of this effect is unclear.

Key words: Opium, alloxan, biochemistry parameters and rats

INTRODUCTION

Opium (**poppy tears**, *lachrymal 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 opiates for medicinal use and for the illegal drug trade. The latex also contains the closely related opiates, codeine and thebaine and non-analgesic 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 characteristic odor 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 anesthesia[3]. 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 diabetics[6]. 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 patients[7]. Opium is generally absorbed after oral administration and undergo hepatic metabolism, and rapidly cleared from the blood and stored in kidney, liver, brain, lung, spleen, skeletal muscle, and placental tissue. Most of

the opium metabolites are excreted through the kidneys[8]. Chemical mechanisms of the selective action of alloxan inhibit the glucokinase enzyme originally extracted from the liver and present in pancreas 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 cytotoxic[9]. It was reported that opium used for the treatment of alloxanoised rats, which, restores some of the normal function 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 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 carried out in accordance with the guide on the care and use of laboratory animals approved by the committee of Assuit University. It was carried out in the period from January 2015 to August 2015. The animals were housed in cages provided with rice husk as building materials and kept under ambient temperature of 23 ± 2°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, diabetic animals 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 creatinene), 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 Trinder[13]. The blood glucose levels[14] and Insulin[11] 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 Doumas[12], Liver functions (Alanine Aminotrasferase (ALT) and Aspartate Aminotransferase (AST) were determined by Reitman and Frankel[15], kidney functions (Urea by Patton[16] and Creatinine determined by Henry[17]), while Cholesterol and Triglycerides were determined by Lukenes[18].

STATISTICAL ANALYSIS

The results are expressed as mean ± 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 a 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 level 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 plasma urea Level of the diabetes (42±3 mg/dl) compared to control group (16.9±2.1 mg/dl) p-value<0.05 (0.029).

The results showed that, a significant increase in, 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 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 Azod[19], 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 (µu /ml)	Glucose (mg/dl)	Insulin (µu /ml)	Glucose (mg/dl)	Insulin (µu /ml)
3 days	98 ± 3	16.6 ± 2	253 ± 7	6.2 ± 2	218 ± 6	7.6 ± 5
7 days	94 ± 2	16.9 ± 6	338 ± 6	2.9 ± 3	210 ± 7	7.9 ± 3
10 days	95 ± 4	16.9 ± 5	442 ± 5	1.6 ± 2	186 ± 3	10.6 ± 2
15 days	100 ± 2	16.4 ± 3	Dead	Dead	138 ± 4	12.8 ± 6
20 days	99 ± 6	16.5 ± 2	Dead	Dead	179 ± 4	10.8 ± 4
25 days	96 ± 5	16.8 ± 4	Dead	Dead	165 ± 3	11.4 ± 8
30 days	97 ± 3	16.5 ± 2	Dead	Dead	128 ± 5	13.2 ± 2

Alloxanoised animals treated with opium showed lower values of serum total protein (9.3±02 mg/dl) p-value<0.05 (0.042), which agree with Mehdi Mahmoodi[20], who found that addicted diabetic males showed lower levels of total protein comparable to non-addicted diabetic animals. The results showed that, there was a decrease level of cholesterol and triglycerides, (75±4 mg/dl) and (199±6 mg/dl) p-value<0.05 (0.039), p-value<0.05 (0.035) respectively; which didn't agree with Azod[19], 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 the case of cholesterol, there was agreement with Mahmoodi[20], who found that there was a lower level of total cholesterol compare to non-addicted diabetic animals.

Results clear that, there was a decrease level of AST and ALT, (48±3 U/l), (44±7U/l) p-value<0.05 (0.045), p-value<0.05 (0.038) respectively, which didn't agree with Kharchenko[21], who demonstrated, opium addiction caused liver damages, and increased

the activity of AST, ALT, but this agreement with Kharchenko[21] in case of ALT, compare to non-addicted diabetic animals.

Creatinine and urea in addicted diabetic animals were higher (6.5±0.25 mg/dl) and (73±0.43 mg/dl) p-value<0.05 (0.045) p-value<0.05 (0.035), respectively, compared to non-addicted diabetic animals, which didn't agree with our findings Helmstädter[22], 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± 0.8*	42 ± 3*	4.2 ± 0.32*	78 ± 5*	71 ± 6*	233± 4	85 ± 2
Addicted Diabetic rats	9.3 ± 0.2*	73 ± 0.43*	6.5 ± 0.25*	44 ± 7*	48 ± 3*	199 ± 6*	75 ± 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.

References

1. "Define Opium at Dictionary.com". http://dictionary.reference.com/. Reference.com. Retrieved February 28, (2015).
2. "Opium definition". (2015). Drugs.com. Retrieved February 28.
3. Paul L. Schiff, Jr. (2002). "Opium and its alkaloids". *American Journal of Pharmaceutical Education*. Retrieved 2007-05-08.
4. Fatemi S.S., Hasanzadeh M., Arghami A., Sargolzaee M. R. (2008). "Lipid Profile Comparison between Opium Addicts and Non-Addicts". *J Tehran Univ Heart Cent*; 3(3): 169-72.
5. Shirani S., Shakiba M., Soleymanzadeh M., Esfandbod M. (2010)."Can opium abuse is be a risk factor for carotid stenosis in patients who are candidates for coronary artery bypass grafting". *Cardiol J*; 17(3): 254-8.
6. Karam G. A., Reisi M., Kaseb A. A., Khaksari M., Mohammadi A., Mahmoodi M. (2004). "Effects of opium addiction on some serum factors in addicts with non-insulin-dependent diabetes mellitus". *Addict Biol*.; 9(1):53-8.
7. Sadeghian S., Darvish S., Davoodi G., Salarifar M., Mahmoodian M., Fallah N. (2007). "The association of opium

with coronary artery disease". *Eur J Cardiovasc Prev Rehabil*.; 14(5):715-7.

8. The Merck Veterinary Manual-Opiates, (2006). Merck & co., Inc., Nj. USA.
9. Lenzen S., Panten U., (1988)." Alloxan: history and mechanism of action". *Diabetologia*, 31, 337.
10. Sridevi S., Chary M., Krishna D. (2000). "Anthihyperglycemic effect of Ipomoea Carnea levels in streptozotocin induced diabetic analysis ".*Pharmacology*, 23, 309.
11. Nicole S., Noel J., Anthony R., Gareth D., Graham A. (2001). "Effect of a series of novel sulphonylthioureas on glucose tolerance in the obese fa/fa Zucker rat", *Pharmacology and Physiology*, 28, 386.
12. Mcletchie N., Coll. J. R. (2002). "Alloxan diabetes: a discovery, albeit a minor one ", *Physicians Edinb*, 32, 134.
13. Trinder P. (1969). "Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor ".*Ann. Clin. Biochem*., 6, 24.
14. Sridevi S., Chary M., Krishna D. (2000). "Development and Evaluation of Transdermal Drug Delivery System Using New Polysaccharides ", *Pharmacology*, 23, 309.

15. Reitman S., Frankel S., (1957)." A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases", *Amer*, J., *Clin*. *Path*., 28, 56.

16. Patton C. J., Crouch S. R. (1977). "An improved determination of ammonia in kjeldahl digests and acidic solutions with a buffered berthelot reaction". *Anal. Chem*., 49, 464.

17. Delanghe, Joris R. and Marijn Speeckaert (2011)."Creatinine determination according to Jaffe—what does it stand for?", *Nephrology Dialysis Transplantation Plus* (0): 1–4. Retrieved October 19, 2012.

18. Lukenes F.D.W., (1948). "Effect of honey bee venom-apis-mellifera on hyperglycemia and hyperlipidemia in alloxan", *Physiological Reviews*, 28, 304.

19. Azod, L., Rashidi, M., Afkhami-Ardekani, M., *et al*. (2008). "Effect of opium addictionon diabetes". *American Journal of Drug Alcohol Abuse*, 34, 383-388.

20. Mehdi Mahmoodi1, Seyed-Mostafa Hosseini-Zijoud, Javad Hosseini, Ahmadreza Sayyadi, Mohammadreza Hajizadeh, Gholamhossein Hassanshahi, Behrooz Hashemi. (2012) "Opium withdrawal and some blood biochemical factors in addicts' individuals". *Advances in Biological Chemistry*, 2, 167-170

21. Kharchenko, N.K., Synyts'kyi, V.N. and Kovtun, T.V. (2001) "Comparative analysis of the effects of alcoholism and opium addiction on liver function". *Fiziol Zh*, 47, 81- 86.

22. Helmstädter, A., (2007) Antidiabetic drugs used in Europe prior to the discovery of insulin. *Pharmazie*, 62, 717-720.