World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Effect of protease extracted from *Echis carinatus sochureki* venom on some biochemical parameters of normal and diabetic male rats

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Received: 07-11-2014 / Revised: 17-11-2014 / Accepted: 25-11-2014

ABSTRACT

The present study was designed to determine the effect of protease enzyme extracted from *Echis carinatus sochureki* venom (So-called "Said Dakheel snake") in two doses (0.05 and 0.1 ml/animal /day) on some biochemical parameters of normal and diabetic male rats. The process of enzyme purification was carried out by using techniques of gel filtration chromatography, dialysis and ion exchange chromatography. The treatment of normal and diabetic male rats with two doses of protease was found to cause a reduction in the glucose level compared with control group. Also, there was a significant decreasing ($P<0.05$) in concentrations of serum total protein, albumin and HDL, while there was a significant increasing in the concentrations of each of the MDA, total cholesterol, triglycerides, LDL, VLDL, ALT, AST.

Keyword: *Echis carinatus*, Protease, Biochemical Parameters, Diabetes.

INTRODUCTION

Snake venoms are rich sources of bioactive polypeptides and proteins components. Some of these components, such as enzymatic activities, include proteinases, phospholipases A_2, nucleotidases, phosphodiesterases and L-amino acid oxidases. Other snake venom proteins and polypeptides do not show any enzymatic activity and are described as "non-enzymatic proteins" [1,2,3]. The effect of venom and intensity is determined by a number of factors. The toxicity of venom which determined by the enzymatic compounds are usually determined by the number of LD50. This procedure is based on the test which is injected into large groups of mice varying quantities of venom. It was found that he dose in which 50% of the mice die in 24 hours is the LD50. There are also other factors related to the bite itself, for example, site of the bite, proximity to major blood vessels, the penetration of one or both fangs, and the amount of venom injected. In addition, the health of the snake and the time since the last time the venom used also affect the outcome. The influencing factors associated with the human who keeps the bite such as his\her weight, age, health and mental state [4]. *Echis carinatus* is considered one of the most dangerous kinds of poisonous snakes in the Middle East and Central Asia, especially the Indian subcontinent. They youngest members of the four adult snakes are responsible for causing them on snake bite cases and deaths. This is because of various factors, including their distribution in areas of high population density, and un-cleaned nature [5]. Their Size range between 38 and 80 cm (15 to 31.5 inches) in total length (body+tail), but usually no more than 60 cm (23.5 in) [6], The head distinct from the neck, and the snout very short and rounded. The nose between the three shields and the head is covered with small scale skeeled which found above the eye enlarged in some cases. There are 9-14 interocular scales between the eyes or across the top of the head, and about 14-21 circumorbital scales. In addition, there are 1-3 rows of scales separate supralabials from the eye. And also, there are 10-12 supralabials, usually the fourth is the largest, and 10-13 sublabials [6]. On the other hand, in the Midbody there are 25-39 rows of dorsal scales that are keeled scales with apical pits; on the flanks, these have serrated keels, there are 143-189 ventral scales which round and cover the full width of the belly, The subcaudals are undivided and number 21-52, and the anal scale is single [6]. Proteases are groups of enzymes that have the ability to hydrolyze peptide bonds in proteins and polypeptides. They are classified as serine protease, cysteine protease, aspartic Proteases and metallo protease [7]. Proteases are used in the manufacture of detergents, food, leather, photography and the

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meat industry [7]. Diabetes mellitus is one of the most common and dangerous problems in our country, Iraq, that affects all body systems. For example, its effect on the peripheral nerves leads to severe diabetic neuropathy, which produces neuropathic pain, sensory loss, weakness and functional deficits. Diabetes is a metabolic disorder characterized by high glucose levels in the blood resulting from defects in insulin secretion, action of insulin, or both [8]. The present study aimed to investigate the effect of protease extracted from *Echis carinatus sochureki* venom on some biochemical parameters of normal and diabetic male rat**.**

MATERIALS AND METHODS

Venom collection: The venom collected from *Echis carinatus sochureki* (So-called "Said Dakheel snake") from "Said Dakheel" area of Thi-Qar province/ South of Iraq, and put in the laboratories of the animal house in Biology Department/ College of Science/ Thi-Qar University. Then it dried by using lyophilization. The weight of the venom was about 24mg.

Isolation and purification of protease: The extraction of protease enzyme from lyophilized crude venom of *Echis carinatus* according to [9].

Protease assay: The activity of protease was determined by the method described by [10, 11].

Protein determination: The concentration of protease was determined according to [12] using BSA as a standard.

Experimental animals: 36 adult male rats weighting (250-370g) were used in present study. Animals were housed in the animal house of Biology department, Sciences College, in Thi-Qar University, Iraq. Animals were housed in plastic cages and metallic clasp covers with wooden chips under standard laboratory conditions 12h light: 12h dark photo period (LD) at 20ºc -25ºc [9]. The animals were divided in to six groups (each group of six animals), as following: group A was injected (I.P.) with 0.1ml of normal saline (0.9%Nacl) daily for 5 days, group B (diabetic group) was injected (I.P.) with 125mg/kg of alloxan and left untreated to the end of the experiment, groups C and D were injected (I.P.) with 125mg/kg alloxan, then treated with (0.05 and 0.1 ml/animal /day) respectively of protease partially purified daily for 5 days. Groups E and F were injected (I.P.) with (0.05 and 0.1 ml/animal /day) respectively of protease partially purified daily for 5 days. After 24 hours of last inject, the animals drugged with diethyl ether. The blood was drawn from each animal of experimental groups by heart puncture method. 2ml of blood were put in the tubes without EDTA and centrifugation at 3000 rpm for 15 min for obtained serum in order to determine the biochemical tests. The biochemical parameters were measured.

Induction of diabetes: The induction of diabetes experimental in laboratory male rats was done by holding food for a period of (12 hours) approximately. Then the animals were injected (I.P.) with 0.5ml (125 mg / kg) of alloxan monohydrate for each animal. After that, the animal injected with (1ml) of glucose (20%), and the drinking water was replaced with a solution of glucose (5%) for 24 hours to prevent the occurrence of severe hypoglycemia resulting from damage to cells of the pancreas, which may lead to kill the animals. After 7 days of the injection, the animals were showing signs of extreme fatigue and frequent urination which refer to the diabetes [13].

Statistical analysis: Statistical analysis was done by using the software SPSS version 14. The results were expressed as mean ± standard deviation (mean ± SD), the value of probability was ($P<0.05$).

RESULTS AND DISCUSSION

The result in table (1) showed a significant increase ($P<0.05$) in the glucose level of the group B compared with groups A, C, D, E and F. The significant elevation in plasma glucose level of injected rats by alloxan could be due to damage the insulin secreting pancreatic β-cell, which results to decreasing in endogenous insulin release [14, 15]. Also the result showed a significant decrease ($P<0.05$) in the glucose level of groups C, D, E, F compared with group B. This attributed that protease could play a role in repairing the damage of pancreatic beta cells and promote insulin synthesis thereby which decrease the level of plasma glucose [16]

Table (1) Effect of protease extracted from *Echis carinatus sochureki* venom on the Glucose level of normal and diabetic male rats:

The treatments	Glucose level (mg per dl) Mean±S.D.
A	108.86^{d} ±4.23
B	235.50^{a}±24.84
C	87.36^{e}±18.02
D	75.25^{e}±3.88
E	175.29^{b}±4.88
F	143.67^{c}±6.08
R.L.S.D.	20.86

The result in table (2) showed a significant increase (P<0.05) in the MDA level of group B compared with groups A, C, D, E and F**.** This increment may be return to that MDA level has been associated with increase of glucose level, Since the high level of glucose in the blood leads to the free radicals generation that lead to increase oxidative stress for fats [17]. While groups E, F appeared a significant decrease (P<0.05) of MDA level compared with groups B, C, D. Therefore, protease is a natural antioxidative agent, which protects against oxidative damage by scavenging excess free radicals, reducing lipid peroxidation, and affecting components of the antioxidative defense system [18].

Also the result in table (2) showed a significant decrease (P<0.05) in the total protein and albumin level of groups B, C, D, E, F compared with group A. This attributed to that diabetics was inhibition of oxidative phosphorylation which leads to decrease in protein synthesis, increase in catabolic processes and reduction of protein absorption [19]. Also the albumin levels in hepatic were found to be decreased in diabetics [20]. As well as it might be assumed that the reduced levels of these serum constituents by protease could be due to disturbances in renal function and hemorrhages in some internal organs. In fact, increased vascular permeability and hemorrhages in vital organs due to the toxic action of various snake venoms were described by the references [21, 22].

Table (2) Effect of protease extracted from *Echis carinatus sochureki* venom on the MDA, total protein and albumin levels of male rats:

The treatments	MDA level (nmole/ml) Mean ±S.D.	Total Protein level (g/100ml) Mean± S.D.	Albumin level (g/100ml) Mean±S.D.
A	21.82^{e}±1.11	8.97^{a}±0.31	5.96^{a}±0.24
B	49.86^{a}±1.29	8.46^{b}±1.94	5.43^{b}±1.06
C	36.78^{c}±1.20	8.03^{c}±0.43	5.09^{c}±0.23
D	42.90^{b}±1.94	7.82^{c}±0.28	4.65^{d}±0.31
E	29.62^{d}±2.45	7.37^{d}±0.24	4.22^{e}±0.22
F	22.53^{e}±1.19	6.54^{e}±0.37	3.98^{e}±0.37
R.L.S.D.	4.84	0.44	0.33

The result in table (3) showed a significant increase (P<0.05) in total cholesterol, triglyceride, LDL, VLDL levels in groups B, C , D, E and F compared with group A. This attributed to that Diabetes causes an increase in the cholesterol, triglycerides, LDL and VLDL [23]. High levels of total cholesterol in blood are major coronary risk factors. The abnormal high concentration of serum lipids in the diabetic subject is due mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase, While HDL level decreased significantly (P<0.05) because that Hyper triglyceridaemia usually accompanies decreased HDL, which is also a prominent feature of plasma lipid abnormalities seen in diabetic subjects [24, 25, 26]. The low level of HDL, which exerts anti-atherogenic and antioxidative effects when present in sufficient amounts, is a key feature of NIDDM (also known as type 2 diabetes

mellitus). The increases in serum cholesterol and level of injected rats by purified protease observed in the study could be due to increase the hepatocytes damage. As a result, it becomes unable to phosphorylate the increasing amounts of fatty acids, hence leading to fatty liver and alteration of cell membranes of tissues [27]. The mechanism responsible for hyper triglyceridaemia may be due to an increased hepatic secretion of VLDL and a delayed clearance of TG-rich lipoproteins, which might mainly be due to increased levels of substrates for TG production, free fatty acids, and glucose.

Table (3) Effect of protease extracted from *Echis carinatus sochureki* venom on the lipid profile of normal and diabetic male rats:

The treatments	Total Cholesterol level (mg/dl) Mean± S.D.	Triglyceride level (mg/dl) Mean± S.D.	HDL level (mg/dl) Mean±S.D.	LDL level (mg/dl) Mean±S.D.	VLDL level (mg/dl) Mean±S.D.
A	2.57^{d}±0.08	1.12^{d}±0.12	3.99^{a}±0.26	1.02^{c}±0.26	0.25^{d}±0.02
B	3.23^{b}±0.74	1.67^{b}±0.39	2.52^{b}±0.28	1.50^{a}±0.33	0.53^{b}±0.07
C	2.99^{c}±0.06	1.37^{c}±0.22	2.33^{b}±0.15	1.31^{b}±0.17	0.35^{c}±0.04
D	3.01^{c}±0.09	1.61^{b}±0.12	2.28^{b}±0.17	1.34^{b}±0.22	0.39^{c}±0.02
E	3.47^{a}±0.09	1.92^{a}±0.35	1.66^{c}±0.19	1.59^{a}±0.17	0.58^{ab}±0.10
F	3.51^{a}±0.15	1.98^{a}±0.14	1.43^{d}±0.11	1.64^{a}±0.22	0.64^{a}±0.02
R.L.S.D.	0.118	0.24	0.21	0.24	0.06

The result in table (4) showed a significant increase ($P<0.05$) in AST and ALT levels in groups B, C, D, E and F compared with group A. This increment might be due to the observed increment in the aminotransferases (AST and ALT) levels in the liver of alloxan-treated animal [28], and the cellular damage in the liver caused by alloxan-induced diabetes. On the other hand, the injection of protease caused a significant rise in serum AST and ALT in rats which were accompanied with brutal injuries and necrosis of hepatocytes as well as a nephrotoxic action of the venom as reported by the reference [29].

Table (4) Effect of protease extracted from *Echis carinatus sochureki* venom on the ALT and AST levels of normal and diabetic male rats:

The treatments	AST activity (U/L) Mean±S.D.	ALT activity (U/L) Mean±S.D.
A	133.6^{e}±3.50	25.34^{d}±2.25
B	285.41^{c}±78.49	45.13^{b}±10.90
C	170.76^{d}±18.96	36.46^{c}±2.67
D	179.82^{d}±2.36	39.84^{c}±1.75
E	340.80^{b}±12.46	53.92^{a}±2.49
F	368.02^{a}±11.41	57.98a±3.32
R.L.S.D	27.12	4.67

CONCLUSION

It was found that the protease extracted from *Echis carinatus sochureki* venom has an impact on some biochemical parameters of normal and diabetic male rats. Also we have observed the role of protease in reducing the levels of glucose, albumine, total protein, HDL, while raising the levels of MDA, total cholesterol, triglyceride, LDL and VLDL in blood of rats.

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