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Ameliorative Effect of Chitosan on Nicotine Toxicity in Diabetic Rats

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Abstract:

Chitosan (COS) is a natural product. It used as a dietary fiber. We explored the impact of chitosan administration in case of nicotine toxicity in adult healthy and corresponding diabetic male rats. Eighty mature male albino rats, weighing 190±10 g, were distributed into two sections healthy and diabetic section. Each section separated into 4 groups each one includes 10 rats, group1: control group, group2: orally administrated, COS, group3: administrated nicotine and group4: administrated both COS and nicotine. The experiment lasted 6 weeks. Diabetes induction with STZ caused significant increase in diabetic biomarker, serum glucose level and HOMA-IR. With a strong decline in insulin, liver glycogen as well as in liver glucokinase enzyme activity. Nicotine worsened the diabetic biomarkers. COS administration caused an ameliorative effect on these diabetic biomarkers that caused by diabetes alone or with nicotine. Diabetes and nicotine caused increase in TAGs, TC, LDL-C, VLDL-C, RF and AI with diminished HDL-C level. COS significantly improved the lipids profile in healthy and diabetic groups. Diabetes induction as well as nicotine injection increased ALT and AST activities and decreased the serum albumin, while COS improved the effects of nicotine and diabetes. Diabetes increased serum creatinine, urea and urine albumin. Also, decreased urine urea and creatinine. Nicotine worsened the kidney function tests in both healthy and diabetic rats. COS improved the renal function. Diabetes and nicotine decreased SOD and increased MDA activities. COS increased SOD and decreased MDA. Diabetes and nicotine increased the DNA fragmentation. COS protected the DNA from damage. Finally, the study demonstrated that COS have antidiabetic, antioxidant and antitoxic effects.

Keywords: Diabetes mellitus (DM), Nicotine, Chitosan (COS), antioxidant, DNA damage.

1. INTRODUCTION

Diabetes mellitus (DM) and Cigarette smoking are from the major threat on global health. They both lead to cardiovascular disorders and have a major effect on the absolute risk of death from their co-occurrence. [1]. Diabetes mellitus is a condition of increasing blood glucose level typically along with glucosuria [2] it is a metabolic

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disorder in which insulin efficacy or insulin distribution to tissues is decreased. This occurs due to deficiencies in insulin secretion, insulin action or both. It classified to type 1 diabetes (T1DM) and type 2 diabetes (T2DM). The abnormal glucose homeostasis expressed as hyperglycemia and also affects the metabolism of fat and protein [3,4] The morbid characteristic of DM includes the vasculature contributing to micro-vascular and macro- vascular problems [5]. Prolonged hyperglycemia is linked with long-lasting damage and deterioration of different organ systems affecting the skin, nerves, kidneys and heart in particular. This effect of hyperglycemia due to oxidative stress increases free radical, leading to the rise of Advanced glycation End products (AGEs) that has been formed via the non-enzymatic glycation of plasma [6].

Tobacco and tobacco smoke contain approximately 4000 harmful substances. One cigarette a day provide 25mg tar which contain different cyclic and non-cyclic organic compounds; benzidine (30 nanograms) and nitrosamine (250 nanograms) and others, in addition of the highly active and toxic nicotine (2mg) all readily absorbed [7]. Nicotine is the extremely active compound that acquired across the active and passive smoking. Nicotine has a number of biological side effects, including atherosclerosis, hypertension and pulmonary fibrosis, which can contribute significantly in tobacco-induced disease pathogenesis. Glomerular damage in acute nephritis worsens in addition to nicotine and facilitates the development of human mesangial cells from the extracellular matrix [8,9] Nicotine has been reported to have a variety of biological consequences, including atherosclerosis and lung diseases, which can play a vital role in pathogenesis of tobacco-related ills.

Chitosan (COS) is a chitin-derived natural product. Chitin is the second plentiful natural compound after cellulose [10]. It discovered by [11] he named it “chitine modifiée”, it is a heteropolysaccharide involving of two monosaccharides connected by β-(1→4) glycosidic bonds, N- acetyl- D glucosamine and D-glucosamine. COS used in many food industry applications and as a dietary fiber in baked foods approved by the Food and Drug Administration [12]. Owing to its essential biological plus biochemical features, for instance biodegradability, biocompatibility, bioactivity and polycationic characteristics. It has a wide variety of biological functions, including anti-cancer, anti-inflammatory and anti-diabetic agents [13]. Therefore, this study was conducted to assess the protecting and anti-diabetic impact of chitosan on nicotine toxicity in healthy and corresponding diabetic rats.

2. MATERIALS AND METHODS

2.1. Chemicals

Streptozotocin (STZ) as well as nicotine bitartrate were bought from Sigma for Chemicals Company, Chitosan purchased from Savvy Mix for Distribution and Supply, Cairo, Egypt

2.2. Animals

Eighty healthy mature male albino rats, weighing 190±10 g, delivered from the Breading Unit of the Egyptian Organization for Biological Products and Vaccines Helwan, Egypt. Both rats were allocated alone with constantly monitored environments in metabolic stainless-steel cages, the temperature was 25ºC±5ºC, air humidity 55% ±10% and12/12 hours light /dark period were held.

2.3. Diet

All rats were offered the standard diet set by [14] and tap water *ad libitum*.

2.4. Diabetes induction:

Diabetes induced in rats by STZ given intraperitoneally at a single dose of 40mg/kg body weight (dissolved in citrate puffer, pH 4.5) after 12hours fasting injection. Followed by administration of 5 % fructose solution, three days later, Serum glucose level at the zero time was measured using enzymatic colorimetric method as described by [15]. Serum glucose level reached 200±10 mg/dl.

2.5. Experimental design

Eighty rats divided into two main sections, healthy rats (40 rats) and diabetic rats (40 rats)

Each group contain 10 rats divided as the follows:

Healthy rats' section

Group 1: Healthy control group

Group 2: Healthy rats administrated COS orally at dose of 500 mg/kg body weight.

Group 3: Healthy rats administrated nicotine at dose of 3mg/kg.

Group 4: Healthy rats administrated COS orally at dose of 500 mg/kg body weight and nicotine at a dose of 3mg/kg body weight.

Diabetic rats' section

Group 5: Diabetic control group.

Group 6: Diabetic rats administrated COS as reported.

Group7: Diabetic rats Administrated nicotine as reported.

Group8: Diabetic rats administrated COS and nicotine as reported.

2.6. Sample collection

At the end of the study period (6weeks), All animals have been sacrificed; hepatic portal vein blood samples were obtained. Serum has been isolated at 3000 r.p.m. for 10 minutes at 4°C after blood clotting and maintained for biochemical analysis. Pancreas, liver and kidney are isolated and cleaned, rinsed immediately and washed on filter paper by a saline solution for removal of water and processed for biochemical evaluation.

2.7. Biochemical analysis

Serum fasting glucose level was assessed matching to the enzymatic colorimetric technique as described by [15]. Serum insulin was assessed utilizing insulin ELISA Kit for rats as described by [16]. Meanwhile, homeostatic model assessment of insulin resistance (HOMA-IR) was determined by calculation of [17]. Liver glycogen content along with liver glucokinase enzyme activity were assessed calorimetrically in liver tissue as explained by [18] and [19] respectively. Total cholesterol (TC) and triacylglycerols (TAGs) were measured using enzymatic colorimetric methods described by [20] and [21] respectively. Very low-density lipoprotein cholesterol (VLDL-C) was calculated as described by [22]. Low density lipoprotein cholesterol (HDL-C)was evaluated using enzymatic colorimetric as described by [23], low density lipoprotein cholesterol LDL-C was calculated according to [24]. The atherogenic risk factor (RF) calculated as described by [25]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activity and serum albumin were measured calorimetrically as described by [26] and [27] respectively. Serum and urine urea and serum and urine creatinine levels were measured as described by [28] and [29] respectively. The antioxidant enzyme superoxide dismutase (SOD) and lipid Peroxidation byproduct, malonaldehyde (MDA) were measured in pancreas tissue calorimetrically according to [30] and [31] respectively.

2.8. Evaluation of DNA destruction in the kidney tissue

1 g of specimen smashed were moved to 1 ml of cold puffer sulphate solution (PBS), then extracted and mixed for five minutes. A low-melting agarose blended the colic suspensions (0.8 percent in PBS). The precoated slides were scattered 100 µl of this combination. The coated slides have been tucked into a buffer for 15 minutes (0.045 M TBE and a pH of 8.4 with a 2.5% SDS). The slides have been put in a TBE buffer electrophoresis chamber but were without sodium dodecyl sulphate. The lengths of the comets' tail from the center of the nucleus until the end of the tail were determined with a 40x increase for the number and the comet size. Observations were recorded of Ethidium Bromide -stained for visualizing DNA damage utilizing a 40x objective on a fluorescent microscope matching to [32].

2.9. Statistical analysis

Mean ± Standard deviation (S.D.) from the average result was expressed. The difference between the means was checked by one-way variance analysis using version 19 of the Statistical Package for Social Science (SPSS) program for significance level. While $P < 0.05$ was taken into account as shown by [33] statistically significant.

3. RESULTS

From the result set in table (1), Administration of COS in healthy rats caused non-substantial change in serum glucose in comparison with healthy control group. While administration of nicotine either alone or with COS in healthy rats indicated a major rise in serum glucose. Serum glucose levels in the diabetic control group have been substantially increased. However, the COS administration showed a substantial decrease in serum glucose levels in diabetic rats. In contrast, the mean value of serum glucose level in diabetic rats treated with nicotine either alone or with COS caused a significant increase. Administration of COS caused non-significant change in serum insulin level. In contrast, the administration of nicotine either alone or with COS in healthy rat, caused a major decrement in serum insulin and caused a non-substantial change in the means value of HOMA-IR. Induction of diabetes by injection of STZ caused a large decrease in the amount of serum insulin and caused a condition of insulin resistance appeared as a substantial rise in HOMA-IR in comparison with the healthy control group. While treated diabetic rats with COS resulted in significant increments in insulin level and a substantial decrease in HOMA-IR. Administration of nicotine

either alone or with COS in diabetic rats revealed a major decrement in insulin level, additionally, the administration of nicotine alone or with COS produced no noticeable changes in HOMA-IR. COS administration caused an increase in liver glycogen content. Moreover, there was non-substantial change in liver glucokinase enzyme in healthy treated rats. While administration of nicotine either alone or with COS, the liver glycogen content decreased significantly. Moreover, there was slightly significant decrease in liver glucokinase activity. Diabetes induction caused a significant decrease in liver glycogen content and liver glucokinase activity. Moreover, administration of COS in diabetic group caused a significant increase in liver glycogen content and liver glucokinase. In contrast, nicotine administration either alone or with COS caused a substantial reduction in liver glycogen content. Also, the activity of liver glucokinase has decreased slightly.

From the results of lipids profile in table (2), COS administration induced a substantial decrease in TAGs. While nicotine administration either alone or with COS induced a substantial increment in TAGs. Induction of diabetes produced a substantial rise in TAGs in diabetic control group, COS administration in diabetic rats caused a significant decrement in TAGs. While nicotine administration either alone or with COS in diabetic rats caused substantial rise. COS administration caused a substantial decrement in TC level in healthy rats. However, nicotine administration either alone or with COS in healthy rats caused a significant rise in TC level. Induction of diabetes by STZ as diabetic control group caused a significant ($p<0.05$) rise in TC. COS administration in diabetic rats caused a substantial decrease. While nicotine administration either alone or with COS caused a significant rise. COS caused a significant increase in HDL-C in healthy rats. However, nicotine administration either alone or with COS in healthy rats caused a significant decrease in HDL-C. Diabetes induction caused substantial decrease in serum level of HDL-C. Also, nicotine administration either alone or with COS in healthy rats produced a significant ($p<0.05$) decrements in HDL-C. COS administration caused substantial decrease in serum VLDL-C level. While nicotine administration either alone or with COS caused a significant ($p<0.05$) rise. The level of VLDL-C in the diabetic control rats had a substantial rise. Also, nicotine administration either alone or with COS caused a significant increment in VLDL-C. From the result of LDL-C, COS administration to healthy rat caused a substantial decrease. While the administration of nicotine either

alone or with COS in healthy rats revealed a significant rise in serum LDL-C level. COS in diabetic rats caused a substantial decrease in LDL-C. In contrast the mean value of serum LDL-C level in diabetic rat, administrated nicotine either alone or with COS caused a significant rise. COS administration in healthy rats caused non-remarkable change comparing with the healthy control rats. While nicotine administration either alone or with COS caused significant rise ($p<0.05$), in RF and for AI. The risk factor and atherogenic index for the diabetic control group increased. COS administration caused a significant decrease, while nicotine administration either alone or with COS caused substantial rise in both RF and AI.

From table (3), the effect of diabetes on the liver, the activity level of ALT and AST enzymes demonstrated substantial increases. However, COS administration to diabetic rats produced a substantial decrement in the serum level of ALT and AST activities. Nicotine administration either alone or with COS produced a significant ($p<0.05$) rise. COS administration caused non- important rise in the healthy control group in serum albumin. While nicotine administration either alone or with COS induced a substantial decrease. In the diabetic control rats, a substantial reduction in serum albumin has occurred. COS administration induced slightly significant rise in serum albumin. While nicotine administration either alone or with COS induced slightly significant reduction in serum albumin level.

From the results in table (4), A substantial increase in serum urea and creatinine levels was induced by nicotine injection to healthy rats, either alone or with COS. COS administration in diabetic rats induced a significant reduction in these levels. While nicotine administration to diabetic rats either alone or with COS induced a significant rise. Administration of COS caused non-important change in urine urea and creatinine. In contrast administration of nicotine either alone or with COS in healthy rats, induced a substantial reduction in urine urea and urine creatinine. Induction of diabetes induced a substantial decrease in urine urea and urine creatinine. While treated diabetic rats with COS resulted in slightly substantial rise in urine urea and in urine creatinine. Administration of nicotine either alone or with COS showed significant reduction in urine urea and creatinine and significant increase in urine albumin. Administration of COS in healthy rats caused non-important change in urine albumin. Diabetes induction induced a significant rise in urine albumin in the diabetic control rats. While administration of COS induced slightly significant reduction in urine albumin. In

contrast, administration of nicotine either alone or with COS in diabetic rats induced a significant rise.

From the results in table (5), administration of COS in healthy rats induced a substantial increase in pancreatic SOD. While nicotine administration either alone or with COS in healthy rats showed a significant reduction in pancreatic SOD activities. Induction of diabetes by STZ induced substantial decrease in SOD. However, COS administration to diabetic rats produced a substantial rise in pancreatic SOD. In contrast, nicotine administration either alone or with COS produced significant decrement. Administration of COS decreased the level of MDA. While nicotine administration either alone or with COS to healthy rats induced slightly significant rise in pancreatic MDA level. Diabetes induction increased the pancreatic MDA level. COS administration in diabetic rats induced a substantial decrease. While nicotine administration to diabetic rats either alone or with COS induced a significant rise in pancreatic MDA.

From the results in table (6), and figure (1) Comet assay is a delicate technique for the detection of DNA damage. As the healthy control group recorded (4.28±0.05%). Considering the effect of COS on healthy control group, COS induced a significant reduction in the damage of kidney cell's DNA the percent of damage, Tailed DNA (%), was (3.86±0.07%). In addition, COS substantially risen untailed DNA% that recorded 96.24±0.28% compared with 95.71±1.80% in healthy control group. while tail length (µm), tail DNA% and tail moment mean values were non-remarkable decrements in chitosan group. While nicotine administration in healthy rats either alone or with COS produced a damage in kidney cells DNA the percent of damage (Tailed DNA %) reached to 5.68±0.28%and 5.57±0.28% respectively compared with healthy control rats. In addition, it decreased the levels of comet parameter untailed DNA%. Regarding the tail length (µm), tail DNA%, and tail moment nicotine administration in healthy rats either alone or with COS caused substantial increments. There was a significant rise in the damage of kidney cells DNA due to diabetes the percent of damage DNA. However, COS protected the kidney cells DNA from this damage and induced a substantial reduction in % tailed DNA. Also, COS increased significantly % of untailed DNA. In addition, COS decreased the levels of comet parameters tail length (µm), tail DNA % and tail moment. Nicotine administration either alone or with COS produced a significant rise in tailed DNA%. This resulted in substantial decrease in the untailed

DNA%. In contrast, nicotine administration either alone or with COS caused a significant rise in levels of comet parameters tail length (µm), tail DNA% and tail moment.

Table (1): Diabetic biomarker in healthy and corresponding diabetic groups in case of chitosan and/or nicotine administrations

Groups Parameters	G1	G2	G3	G4	G5	G6	G7	G8
Serum glucose (mg/dl)	**g** **65.00±2.7**	**g** **64.12±0.83**	**e** **77.75±2.60**	**f** **70.76±3.8**	**b** **220.5±3.27**	**d** **140.54±2.45**	**a** **249.10±2.26**	**c** **185.5±1.73**
Serum insulin (mµ/ml)	**a** **32.43±0.32**	**a** **32.48±0.27**	**c** **28.56±0.33**	**b** **30.92±0.64**	**f** **13.10±0.21**	**d** **19.27±0.20**	**g** **11.90±0.59**	**e** **15.12±0.89**
HOMA-IR	**c** **5.20±0.10**	**c** **5.14±0.04**	**c** **5.48±0.06**	**c** **5.40±0.16**	**a** **7.13±0.09**	**a 6.68±0.09**	**a** **7.32±0.16**	**a** **6.92±0.19**
Liver glycogen content(mg/100g tissue)	**b** **49.44±2.16**	**a** **52.38±1.95**	**d** **41.63±0.92**	**c** **44.01±1.29**	**f** **27.05±0.97**	**e** **32.37±1.58**	**h** **21.75±1.00**	**g** **24.26±0.65**
Liver glucokinase (U/L)	**a** **0.24±0.001**	**a** **0.25±0.004**	**b** **0.20±0.003**	**c** **0.22±0.003**	**e** **0.11±0.013**	**d** **0.18±0.010**	**g** **0.075±0.007**	**f** **0.095±0.007**

-Values are represented as mean ± SD

-10 rats for each group

-There is no significant differences between means have the same letters in the same raw for each section ($p<0.05$)

Table (2): Lipids profile in healthy and corresponding diabetic groups in case of chitosan and/or nicotine administration.

Groups Parameters	G1	G2	G3	G4	G5	G6	G7	G8
TAGs (mg/dl)	**g 133.21±1.30**	**h 129.07±1.53**	**e 142.33±2.03**	**f 137.95±3.6**	**c 165.20±3.08**	**d 152.81±2.81**	**a 185.70±2.50**	**b 170.55±1.62**
TC(mg/dl)	**g 118.52±1.38**	**h 112.95±1.40**	**e 151.9±1.77**	**f 140.65±1.49**	**b 180.10±1.55**	**d 162.94±2.72**	**a 189.56±2.36**	**c 173.51±2.67**
HDL-C (mg/dl)	**b 47.52±0.61**	**a 51.15±1.73**	**d 35.89±1.34**	**c 39.81±2.26**	**f 26.53±1.72**	**e 30.17±0.17**	**h 19.24±2.4**	**g 23.01±1.70**
VLDL-C(mg/dl)	**g 26.64±0.26**	**h 25.81±0.30**	**e 28.46±0.40**	**f 27.59±0.72**	**c 33.04±0.61**	**d 30.56±0.56**	**a 37.14±0.50**	**b 34.11±0.32**
LDL-C (mg/dl)	**g 44.36±0.81**	**h 35.99±1.82**	**e 87.55±1.85**	**f 73.25±2.98**	**b 120.71±2.49**	**d 102.53±2.87**	**a 132.62±2.80**	**c 116.39±2.54**
Risk factor (LDL-c/ HDL-c	**g 0.93±0.02**	**g 0.70±0.11**	**e 2.43±0.11**	**f 1.84±0.22**	**c 4.54±0.39**	**d 3.39±0.10**	**a 6.89±0.98**	**b 5.05±0.31**
Atherogenic index(AI)	**g 1.56±0.09**	**g 1.50±0.02**	**e 1.79±0.03**	**f 1.69±0.04**	**c 2.23±0.07**	**d 2.00±0.01**	**a 2.95±0.02**	**b 2.48±0.08**

Values are represented as mean ± SD

-10 rats for each group

-There is no significant differences between means have the same letters in the same raw for each section ($p<0.05$)

Table (3): Liver function tests in healthy and corresponding diabetic groups in case of chitosan and/or nicotine administration.

Groups Parameters	G1	G2	G3	G4	G5	G6	G7	G8
Serum ALT (U/L)	**g 26.71±2.15**	**g 25.37±1.20**	**e 38.35±2.22**	**f 32.63±1.69**	**b 68.45±2.95**	**d 47.79±1.22**	**a 79.44±0.68**	**c 75.36±0.88**
Serum AST (U/L)	**f 40.50±0.29**	**f 36.98±0.58**	**e 54.67±0.44**	**e 52.60±0.23**	**b 77.36±0.38**	**d 62.28±0.29**	**a 85.17±0.18**	**c 82.55±0.76**
Serum albumin (g/dL)	**a 3.12±0.09**	**a 3.14±0.06**	**c 2.68±0.19**	**b 2.79±0.23**	**f 2.27±0.29**	**d 2.62±0.11**	**g 2.08±0.08**	**e 2.14±0.44**

Values are represented as mean ± SD

-10 rats for each group

-There are no significant differences between means have the same letters in the same raw for each section ($p<0.05$)

Table (4): Kidney function tests in healthy and corresponding diabetic groups in case of chitosan and/or nicotine administration.

Groups Parameters	G1	G2	G3	G4	G5	G6	G7	G8
Serum urea (mg/dl)	**g 23.11±1.17**	**g 23.70±1.06**	**e 32.47±2.84**	**f 27.29±1.58**	**b 49.00±2.36**	**d 39.74±1.03**	**a 56.04±2.25**	**c 44.78±2.12**
Serum creatinine (mg/dl)	**g 0.80±0.06**	**g 0.80±0.03**	**e 1.00±0.02**	**f 0.91±0.03**	**c 1.18±0.1**	**d 1.03±0.04**	**a 1.46±0.05**	**b 1.23±0.02**
Urine urea (g/day)	**a 4.34±0.21**	**a 4.35±0.18**	**e 3.77±0.20**	**b 3.98±0.08**	**d 4.12±0.05**	**c 4.18±0.11**	**g 3.69±0.13**	**f 3.75±0.31**

Urine creatinine (g/day)	**a 1.34±0.21**	**a 1.35±0.12**	**c 1.04±0.15**	**b 1.16±0.02**	**e 0.80±0.01**	**d 0.92±0.02**	**g 0.40±0.01**	**f 0.68±0.01**
Urine albumin (g/day)	**g 0.018± 0.0007**	**g 0.017± 0.0006**	**e 0.026± 0.0007**	**f 0.022± 0.0004**	**c 0.034± 0.0007**	**d 0.030± 0.0003**	**a 0.045± 0.0003**	**b 0.040± 0.0008**

Values are represented as mean ± SD

-10 rats for each group

-There are no significant differences between means have the same letters in the same raw for each section ($p<0.05$)

Table (5): Antioxidant and oxidative stress in healthy and corresponding diabetic groups in case of chitosan and/or nicotine administrations

Groups Parameter	G1	G2	G3	G4	G5	G6	G7	G8
Pancreatic SOD (u/g tissue)	**b 50.78±1.09**	**a 55.57±1.45**	**d 35.27±1.33**	**c 42.44±2.55**	**g 24.27±1.19**	**e 31.02±0.64**	**h 20.19±1.64**	**f 27.90±0.49**
Pancreatic MDA (n mol/g tissue)	**g 2.17±0.04**	**h 1.84±0.08**	**e 2.54±0.03**	**f 2.20±0.01**	**c 4.24±0.16**	**d 3.79±0.14**	**a 5.06±0.03**	**b 4.68±0.38**

Values are represented as mean ± SD

-10 rats for each group

-There are no significant differences between means have the same letters in the same raw for each section ($p<0.05$)

Table (6): DNA damage in the kidney tissue in healthy and corresponding diabetic groups in case of chitosan and/or nicotine administration.

Groups Parameter	G1	G2	G3	G4	G5	G6	G7	G8
Tailed (%)	**f 4.28±0.05**	**g 3.86±0.07**	**e 5.68±0.28**	**e 5.57±0.28**	**c 6.67±0.05**	**d 6.06±0.09**	**a 9.53±0.08**	**b 8.07±0.04**
un tailed (%)	**a 95.71±1.80**	**b 96.24±0.28**	**c 94.22±0.05**	**c 94.41±0.05**	**e 93.32±0.31**	**d 93.90±0.15**	**g 90.48±0.04**	**f 91.92±0.04**
Tail length (µm)	**e 1.05±0.02**	**e 1.03±0.018**	**d 1.95±0.075**	**d 1.83±0.037**	**c 2.19±0.24**	**d 1.92±0.19**	**a 3.00±0.018**	**b 2.80±0.04**
Tail DNA (%)	**d 1.10±0.019**	**d 0.90±0.027**	**b 1.85±0.03**	**b 1.79±0.046**	**b 1.81±0.039**	**c 1.62±0.029**	**a 2.60±0.024**	**a 2.45±0.016**
Tail moment (unit)	**d 1.14±0.024**	**d 1.03±0.034**	**c 3.65±0.13**	**c 3.61±0.036**	**b 4.41±0.17**	**c 3.53±0.04**	**a 5.29±0.04**	**b 4.65±0. 11**

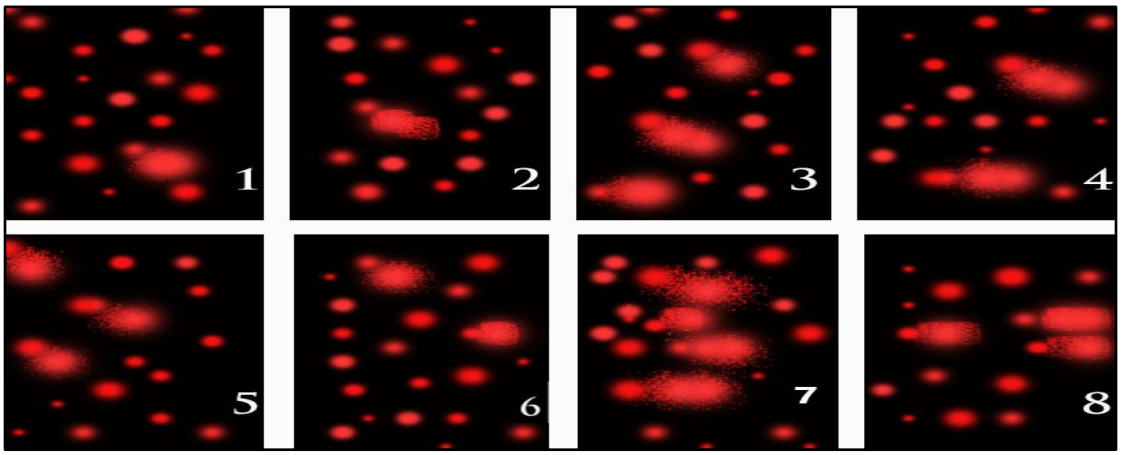

Figure (1): The comets tails lengths were evaluated from the middle of the nucleus to the ending of the tail by 40x increase for the number and determine the size of the comet.

4. DISSCUTION

Diabetes mellitus (DM) is a syndrome characterized by elevated glucose levels induced either by inadequate insulin secretion (Type 1 DM) or insulin action (type 2 DM)). DM is a major health worry the global and is substantial risk factor for cardiovascular, hypertensive and other complications [34]. Nicotine has a negative effect on insulin-secreting β-cells via neuronal nicotinic acetylcholine receptors (nAChRs) on the β-cells of pancreatic islets. NAChRs subunits, such as α-2, α-3, α-4, α-5, alpha-7 and β-2, exist on the surface of islet cells in the pancreas. The β-cell secretion decreased in both acute (60min) and chronic (48 h) exposures of nicotine. The results indicate that nAChRs play a major role in the hormone release regulation of β cells. Furthermore, nicotine levels above 1μmol/L were shown to inhibit insulin secretion in isolated cells of human islets. [35]. Nicotine stimulates sympathetic function, increases catecholamine, adrenocorticotropic hormone, cortisol, prolactin, and beta-endorphin circulating levels, and reduces the amount of estrogen. These results are all antagonistic to the action of insulin. Smoking thus lowers the level of insulin, delays the catabolism of glucose and contributes to its buildup in the body. Nicotine directly increases the resistance of insulin. Nicotine causes insulin resistance associated with a rise in the level of triacylglycerols, as glucose is transformed into triacylglycerols in fatty tissue. Risen serum FFA and triacylglycerols levels induce impairment of the transport of insulin-stimulated glucose in the skeletal muscle. [36] After absorption into the body, chitosan is metabolized into chitooligosaccaride. The antidiabetic effect of chitosan of this study was linked to the fact that it inhibits β cell losses and promotes β-cell production. Chitosan has been found to enhance hyperglycemia, hyperinsulinemia, and hype-rtriacylglycerolemia. Chitosan improves muscle glucose absorption due to increased membrane insertion of the 4 GLUT4, a major skeletal glucose conveyor, and increased glycemic uptakes, including Akt phosphorylation [37]. Liver glucokinase is an enzyme that promotes the phosphorylation of glucose to glucose-6-phosphate. Chitosan treatment has improved the function of this enzyme by rising glucose influx into Pentose Monophosphate in order to minimize high levels of blood glucose. This increases the output of the reduction agent NADPH, which simultaneously reduces the oxidative stress [38]. Our findings are in line with the outcomes of [39] who found that COS treatment to diabetic mice reduced the fasting glucose in comparison to diabetic group. Furthermore, the

performance of insulin tolerance in the diabetic-COS community was substantially better than that of the diabetic group. Besides this, the results of [38] they indicated that the activities of the enzymes which responsible for the carbohydrates metabolism and glycogen content in the diabetic rat's liver diminished compared to the normal rats. The results of [40] revealed that serum glucose control levels were substantially lower compared with diabetic control levels. The influence of COS given orally before a meal at a dose of 500 mg on blood glucose level after eating assessed in health people [41] was seen as a substantial reduction in the blood glucose levels of healthy people. The results of [42] It has been shown that subcutaneous nicotine injection at a dose of 1.5 mg/kg body weight for 4 months in healthy rats induces pathological changes in both exocrine and endocrine pancreatic tissue. Furthermore, there was a marked increase in glucose levels. Our results were consistent with the outcomes of [43] Prolonged exposure to nicotine rising fasting blood glucose levels and HBA1C levels comparison with control animals. The level of insulin in the treated group has diminished dramatically in comparison with control. Insulin level greatly diminished in the nicotine treated group, compared to control. Losing of pancreatic cells and insulin sensitivity that caused by nicotine may lead to hyperglycemia.

As hyperlipidemia is a disorder of diabetes so, they have also elevated the serum levels of (TC), (TAGs), (LDL-C) and (VLDL-C) with decreased the serum level of (HDL-C. These findings are in consistent with the outcomes with [40]. Various pathways contribute to nicotine lipid alteration, as nicotine triggers the sympathetic adrenal system, leading to increased catecholamine secretion, causes lipolysis, and releases plasma-free fatty acids. There is evidence that the liver takes up these free fatty acids, which enhance the synthesis of very low-density lipoproteins [44]. Via many pathways, chitosan lowers plasma lipids, as chitosan positive charge anions are bound to dietary fat negatively charged lipids, preventing fat digestion and reducing fat storage [45]. Chitosan has a major impact on plasma adipocytokines, which greatly reduces the adiposity index, so COS can monitor circulating triacylglycerol levels and fight some inflammatory syndromes and metabolic alterations [46]. In addition, COS treatment raises serum levels of (HDL-C), a cardio- protective particle that contains lipids which removes the excess cholesterol from tissues and delivers it to the liver for excretion [47]. Our results agreed with [48] who resulted that chitosan low molecular weight could markedly reduce serum TC as well as TAGs. In addition, increased LDL-

C is substantially improved, and the HDL-C levels caused by high fat diets in obese rats are decreased. Also [49] resulted that serum and liver TG, TC, and LDL-C levels in the high fat diet group they were considerably higher than those in the standard diet group. Furthermore, [50] They observed that the mean values of TC, LDL-C and TAGs were effectively improved by chitosan. Furthermore, the outcome of [51] showed that the total cholesterol elevated in smoker. In fasting state, lipid profile tests were performed on 143 patients. The findings indicated that level total cholesterol in the group of smokers was greater than in the group of non-smokers [52].

Results from this study indicated that the administration of nicotine in rats has contributed to significant hepatic damage shown by significant imbalance in biochemical liver marker. In addition to nicotine-produced hepatocellular injure and lipid profile defects, it produces oxidative liver damage via rising lipid peroxidation and diminishing tissues' antioxidant. Nicotine and cotinine have a directly intoxication effect on hepatic oxidative stress and lipid peroxidation, as well as DNA damage caused by free radicals caused by mitochondrial respiratory chain disturbance [53]. Chitosan improved and guarded the liver and kidney from the nicotine toxicity and the oxidative stress from diabetes due to its antioxidant properties. It also reduces oxidative stress and improves the intrinsic protection of antioxidants. Hepatocellular damage has been caused by lipid peroxidation. The foraging activity of COS on hydroxyl-radicals prohibits phosphatidyl choline and linoleate from peroxidation thus it preserves the liver [54]. Our outcomes represented those of [55] They showed that chitosan treatment significantly improved the high fat diet by reducing the levels of AST, ALT, ALP in the serum and enhancing the liver histology against liver fibrosis associated with carbon tetrachloride CCl4. The results of [40] showed that the COS treatments in case elevating serum AST levels caused substantial reduction these levels in all groups compared with diabetic control. About the level of (ALT) data, the diabetic control group indicates a substantial rise comparing to normal control. The findings of [56] indicated that nicotine administration caused an increase in some liver enzymes. It also induced lipid peroxidation by influencing the hepatocyte membrane, causing a shift in the permeability of the membrane and lipid degeneration and accumulation in the liver cells. [57] confirmed that the level of ALT and AST were substantial rise in nicotine treated group suggesting that nicotine can cause liver

membrane damage, thus emitting hepatocyte cytosol enzymes and eventually raising the serum enzymes level.

Renal failure is one of diabetes mellitus complications due to the effect of glucose on the renal glomerulus and the oxidative stress produced due to diabetes. Causing alteration in the functions of kidney, rising the serum levels of creatinine and urea. The outcomes of [58] revealed that COS has a reno-protective role against lithium-induced renal toxicity in rats. They resulted throughout orally restored levels of kidney function tests assessed by COS administration to normally in the COS pre-treated group via lowering the nitrogen levels of urea, creatinine and blood urea. Furthermore, [57] resulted that nicotine induced substantial rise in the mean values of urea comparison with control group Therefore, Smoking cigarettes raises the elimination of albumin in urine, reduces the rate of glomerular filtration, induces increment in the occurrence of renal artery stenosis which is associated with increased the mortality in end-stage renal disease patients. Furthermore, the mean creatinine values in the group treated with nicotine dramatically increased compared to the control group. Additionally, [59] resulted that levels of serum uric acid, creatinine and urea were substantially increased in case nicotine administration. [56] proven that nicotine raised serum creatinine in their study animals, which matched with the outcomes of the present study. It was shown that the administration of nicotine risen dramatically the amount of kidney malondialdehyde, blood urea nitrogen, creatinine, and nitrite oxide levels and diminished glomeruli and tissue amount of reducing/antioxidant power level compared to the control group [60]. Also, [61] revealed that nicotine injection for 28 days shown to reduce the glomerular filtration rates and allow creatinine and urea to be stored in the plasma.

β-cell oxidative stress produced by STZ induced DM, which are especially susceptible to oxidative insult due to their relatively low antioxidant levels. It was found that SOD activity had declined, and the level of MDA raised. The study of the [62] indicated that COS administration contributed to a substantial reduction of oxidative stress to values such as that of the control group. Additionally, compared to vitamin E, the antioxidant influence of COS on lipid peroxidation is greater. Furthermore, [63] revealed that COS ready uptake by cells makes it a very promising compound for use as natural antioxidants. COS also greatly reduces serum FFAs and MDA concentrations and allows large antioxidant enzymes, including SOD and CAT, to be published. [64]

reported that COS significantly reduce serum FFAs and MDA concentrations and rise antioxidant enzymes activities as SOD and glutathione peroxidase, implying that COS controlled the antioxidant enzymes activities and diminished lipid peroxidation. In a study conducted by [65] by administering COS as a preventive agent against zinc toxicity, it was reported that the COS reduced the MDA levels that increased by zinc-induced oxidative stress. [66] observed that there was a greatly rise in SOD activity in CCl_4 + COS treated rats implies the efficient protective method of COS. Also, there results revealed that COS may be associated with diminished oxidative stress and free radical-mediated tissue damage. Our results are matched of those [40] who indicated that COS prohibited the programmed cell death of pancreatic islet. All concentrations of COS can enhance the potential of total antioxidant power and activity of SOD and decreased the level of MDA in pancreatic cells. Moreover, [67] confirmed that maternal dietary supplementation with chitosan increased total SOD capacity and caused a downtrend in the level of MDA on day 110 of gestation. In addition, [68] showed that the antioxidative activity of COS reduced the lipid peroxidation. The expression of antioxidants, including catalase and SOD, can also be controlled by COS. The study of [69] found that lipid peroxidation in rats, which was increased by lead exposure, decreased following COS supplementation, indicating that COS has have potential antioxidant effects on oxidative damage generated by lead exposure. In addition, [68] reported that the anti-oxidative activity of COS inhibited lipid peroxidation. Also, COS regulated the expression of antioxidant enzymes, including catalase and SOD. [70] In nicotine-treated rats, an elevation of MDA was seen in comparison with the control group. An elevated level of MDA can be a result of a decrease in the antioxidant production in the tissues of rats treated with nicotine, thus changing the delicate balance towards reactive oxygen. The widely used measure of oxidative stress and resulting oxidative damage is the lipid Peroxidation of unsaturated fatty acids [71] Exposure to nicotine has shown systematic oxidative stress and has impaired endogenous antioxidant defense mechanisms by down-regulation of catalase and SOD. The study of [72] indicated that antioxidant status profile demonstrated a major alteration in antioxidant enzyme activities after nicotine administration. The biological antioxidant system is deficient as a SOD enzyme, since ROS generation increases. In addition, [43] shown that in the pancreatic extract of the nicotine-treated group the levels of MDA and nitric oxide, the key elements of lipid peroxidation and inflammatory response were

massively reduced by compared with the control group. The long-term administration of nicotine resulted in a substantial reduction in the pancreatic function of SOD. Also, [73] outcome was a substantial reduction in SOD activity and a substantial rise in MDA serum level in the nicotine treated group comparing with the normal control. In addition, [74] showed that nicotine reduced the quantity of serum antioxidants in male mice, contributing to rise the lipid peroxidation.

Our results agreed with [75] study which indicated that COS have prominent protective effects on the DNA damage. Also, the study of [63] showed that COS protect DNA and increase the expression period of genes. In addition, the results of [76] which revealed that the levels of tail length, tail moment, %DNA in tail and olive tail moment were substantially boosted in the peripheral blood of nicotine-treated rats when compared with control group. In another study, a substantial rise in the lipid peroxidation and DNA damage detected in the mice treated with nicotine than in control group. In addition, a major increase noticed in mitochondrial DNA destruction of liver, kidney and spleen [77]. The study of [43] resulted that exposure of nicotine prompt an oxidative stress and tissue damage. Also, indicated that nicotine produced DNA damage. [78] revealed that nicotine induced generation of free radicals and lipid peroxidation. It alters DNA membrane proteins, and produces cell destruction, which are the main causes of weight and kidney index loss. In the study of [79] conducted that nicotine induced oxidative stress and caspase-mediated cell death on the kidney cells.[57] Proved that the free radicals induced by nicotine metabolism trigger lipid peroxidation and reaction to DNA and membrane proteins, thereby causing cell damage. Diabetes increased the DNA damage of the kidney cell's due to the effect of hyperglycemia effect on kidney glomerulus [40]. The lipid deposition in the kidney of diabetic rats has been boosted with nicotine, indicating lipid metabolism disruption due to oxidative stress. Further lipid deposition due to exposure to nicotine has shown that the oxidative stress in DM increases further. A growing accumulation of lipids in the kidney leads to cell damage and diabetes nephropathy [56]. COS has been shown to display antioxidant properties by pathways either free radical foraging or antioxidative enzyme expression enhancement. It has a good scavenging impact against radicals of hydroxyl and superoxide and a weak scavenging function against alkyl radicals [37].

5. Conclusion

Our findings have demonstrated the ability of chitosan as antidiabetic agent due to its ability to modulate numerous biochemical pathways in glucose metabolism and lipids profile. Also, it can improve liver and kidney functions in addition to its antioxidant activity. Moreover, our results shed some light on the toxic effect of nicotine that antagonized insulin action, alter lipid metabolism and increase oxidative stress status.

6. References

[1] S. Yuan, and S. Larsson, "Causal relationship between cigarette smoking and type 2 diabetes mellitus: A Mendelian randomization study", Sci Rep 9,(2019) 19342.

[2] M. Sharofova, P. Nuraliev, S. Sukhrobov, Sagdieva and V. Dushenkov, "Can Avicenna Help Manage the Diabetes Epidemic in Central Asia", Central Asian Journal of Medical Sciences; vol. 3(3):(2017) 200-220.

[3] M. Dwivedi, and A. Pandey, "Diabetes Mellitus and Its Treatment: An Overview", Journal of Advancement in Pharmacology1(1) :(2020)48-58.

[4] P.Giannoulaki, E. Kotzakioulafi, M. Chourdakis, A. Hatzitolios, T. Didangelos, "Impact of *Crocus sativus* L. on Metabolic Profile in Patients with Diabetes Mellitus or Metabolic Syndrome: A Systematic Review", Nutrients12:(2020)1424-1441.

[5] R. Hameed," Relationship Between Smoking and Diabetic Microvascular", Sci.Int.(Lahore),31(1): (2019) B,9-13.

[6] S. Aggarwal, D. Khandelwal, D. Dutta, S. Kalra, and Y. Balhara, Diabetes and Smoking: The Burden of Evidence, Rodriguez-Saldana J. (2019). Springer, Cham.

[7] M. A. Sadek, "Biochemical and nutritional overview on cancer" professional con. Type species (2020) 86-89.

[8] T. Volkan, M. Soycan, Y. Banu, Y., Candeger, and Gulinnaz, "The effect of long-term nicotine exposure on bone mineral density and oxidative stress in female albino rats" Arch Gynecol Obstet; 287:(2013)281-276.

[9] K. Baraona, L.Julie J. Daniels, and L. Daniel, "Tobacco Harms, Nicotine Pharmacology, and Pharmacologic Tobacco Cessation Interventions for Women.J of midwifery and women health", 62(3): (2017) 253-269.

[10] T.Gutiérrez, Chapter 8: Chitosan applications in textile and food industry. In: Ahmed S, Ikram S (eds) Chitosan – derivatives, composites and applications, (2017) Scrivener Publishing LLC, Wiley.

[11] M.Rouget, "Des substances amylacées dans les tissusdes animaux, spécialement des articulés (chitine)", Comp. Rend 48: (1859)792-795.

[12] N.Kahya, "Water Soluble Chitosan Derivatives and their Biological Activities. A Review", Polym Sci5:(2019)1-11.

[13] S. Kim, "Competitive Biological Activities of Chitosan and Its Derivatives: Antimicrobial, Antioxidant, Anticancer, and Anti-Inflammatory Activities", International Journal of Polymer Science (2018). 1-13

[14] P.Reeves, F. Niesen, and G. Fahey, J.R.AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet", Am. J. Nutr., 123:(1993) 1939 -1951.

[15] P.Trinder, "Enzymatic determination of glucose" Ann Clin Biochem; 6: (1969)24-26.

[16] B. Frier, J. Ashby, I. Nairnand J. Bairs, "Plasma insulin, C-peptide, and glycogen concentrations in patients with insulin independent diabetes treated with chlorpropamide", Diabetes Metabolism journal 7:(1981)45-49.

[17] M.Stumvoll, G. Perriello, C. Meyer, and J.Gerich, "Role of glutamine in human carbohydrate metabolism in kidney and other tissues", Kidney Int; vol.55: (2001)778–792.

[18] N.Carrol, R. Lonhely , and J. Roe, "The determination of glycogen in liver and muscles by use of Anthrone reagent ", J. Biol .Chem., 220:(1965)583-593.

[19] A. Pakoskey, E. Lesher, and D. Scott, "Hexokinase of *Escherichia coli*. Assay of enzyme activity and adaptation to growth in various media", *J.* Gen. Microbiol 38:(1965)73-80.

[20] C. Allain, L. Poon, S. Chan, W. Richmond, P. and Fu, "Enzymatic Determination of Total Serum Cholesterol" Clinical Chemistry20 (4): (1974)470–475.

[21] P .Fassati, and L.Prencipe," Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide", Clin. Chem., 28: (1982). 2077.

[22] D. Arcol, "Separation of high-density lipoproteins and determination of cholesterol and phospholipids bound to those fractions" European Heart J15: (1989) 121-124.

[23] M. Lopez-Virella, P. Stone, S. Ellis, and J. Colwell, " Cholesterol determination of high-density lipoprotein separated by three different methods", Clin, Chem23: (1977)882-884.

[24] W. Friedewald, R. Levy, and D. Fredrickson"Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge", Clinical Chemistry; 18(6):(1972)499–502.

[25] M. Notarnicola, D. Altomare, M. Correale, E. Ruggieri, B. D'Attoma A. Mastrosimini, V. Guerra, and M. Caruso, " Serum Lipid Profile in Colorectal Cancer Patients with and without Synchronous Distant Metastases", Oncology 68 (2005)371–374.

[26] A. Reitman, and S. Frankel, "A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases", Amer J. Clin. Path., vol., 28: (1957)56-63.

[27] B .Doumas, W. Watson, and H.Biggs, "Albumin standards and the measurement of serum albumin with bromcresol green",Clin. chim. Acta 31: (1971) 87-96.

[28] J. Fawcett, and J.Soctt," A rapid and precise method for the determination of urea", J, Clin., Path. 13: (1960)156 – 159.

[29] K.Larsen, "Creatinine assay by a reaction-kinetic principle", Clin Chim Acta. 41: (1972)209-17.

[30] M. Nishikimi, N. Roaand K. Yogi, "The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen", Biochem Biophys Res Commun 31; 46 (2)(1972)849-54.

[31] K.Satoh, "Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method", Clin Chim Acta; 90(1):(1978):37-43.

[32] N.Singh, M. McCoy, R. Tice, and E. Schneider ," A simple technique for quantitation of low levels of DNA damage in individual cells", Exp: Cell Res., (175): (1988)184-191.

[33] R.Levesque , SPSS programming and data management: A Guide for SPSS and SAS user. Third Edition, United States of America.

[34] C. Rebecca, and M. Danik, "The effects of bioactive compounds on biomarkers of obesity", Functional foods in Health and Disease 5: (2015)365-380.

[35] M. Mossoń, and H. Milnerowicz, "The impact of smoking on the development of diabetes and its complications" Diabetes and Vascular Disease Research14:(2017) 265–276.

[36] G. Papathanasiou, A. Mamali, S. Papafloratos, and E. Zerva, "Effects of Smoking on Cardiovascular Function: The Role of Nicotine and Carbon Monoxide", Health Science Journal 8 (2): (2014) 272-188.

[37] C.Muanprasat, and V. Chatsudthipong, "Chitosan oligosaccharide: Biological activities and potential therapeutic applications", Pharmacology and Therapeutics13(2017)1-80.

[38] K. Prabu, and E. Natarajan, "Antihyperglycemic Effect of Chitosan of Podophthalmus Vigil In Streptozotocin Induced Diabetic Rats", IJPSR 4:(2013)352-359.

[39] J. Zheng, X. Yuanb,G. Chengc, S. Jiaoc, C. Fengc, X. Zhaoa, H. Yina, Y. Duc, and H. Liu, "Chitosan oligosaccharides improve the disturbance in glucose metabolism and reverse the dysbiosis of gut microbiota in diabetic m ice", Carbohydrate Polymers15; 190:(2018)77-86.

[40] E. Abdel-Rahim, A. Fouad, M. Ahmed, Abdel-latif, and W. Magd, "Anti-Diabetic Effect of Chitosan in Alloxan Induced Diabetic Rats. Research Journal of Pharmaceutical", Biological and Chemical Sciences7(5):(2016)-609-616.

[41] S.Jo, K. Ha, K. Moon, J. Kim, C. Oh, Y. Kim, E. Apostolidis, and Y.Kwon, "Molecular weight dependent glucose lowering effect of low molecular weight chitosan oligosaccharide (GO2KA1) on postprandial blood glucose level in SD rats' model", Int J Mol Sci 14:(2014)14214–14224.

[42] M.Hamed, M. El-Shahat, S. Omnia, Erfan, and N.Lashine," A study on the effect of nicotine on the pancreas of albino rat",Eur: J. Anat, vol. 21: (2017)113-118.

[43] A. Bhattacharjee, K. Shilipi, P. Swagata, M Bithin, K. Alak, B. Aranb, and M. Sandip, "Protective efficacy of folic acid and vitamin b12 against nicotine

induced toxicity in pancreatic islet of the rats", Interdiscip toxical 8:(2015)103-111.

[44] S. Bano, and S. Saeed, "Gender Differences in Nicotine Induced Dyslipidemia and Hyperglycemia in Mice", Journal of Basic and Applied Sciences 10:(2014) 33-38.

[45] M. Rizzo, R. Giglio, D. Nikolic, A. Patti, C. Campanella, M. Cocchi, N. Katsiki, and G. Montalto, "Effects of Chitosan on Plasma Lipids and Lipoproteins: A 4-Month Prospective Pilot Study", Angiology65(6):(2014)538-542.

[46] Y. Luo, and Q.Wang, "Recent Advances of Chitosan and Its Derivatives for Novel Applications in Food Science", J Food Processing and Beverages 1(1):(2013)1-13.

[47] H. Huang, L. Hong, P. Chang, J. Zhang, S. Lu, and B.Zheng, "Chitooligosaccharides attenuate Cu2+- induced cellular oxidative damage and cell apoptosis involving Nrf2 activation", Neurotox Res; 27: (2015)411-420.

[48] H. Pan, Q. Yang, G. Huang, C. Ding, P. Cao, L. Huang, T. Xiao, J. Guo, and Z. Su, Hypolipidemic effects of chitosan and its derivatives in hyperlipidemic rats induced by a high-fat diet",Food Nutr. Res; 60,(2018) 31137.

[49] Y. Jiang, C. Fu, G. Liu, J. Guo, and Z.Su, "Cholesterol-lowering effects and potential mechanisms of chitooligosaccharide capsules in hyperlipidemic rats", Food and Nutrition Research 62: (2018)1446 -1461.

[50] S. Bahijri, L. Alsheikh, G. Ajabnoor, and A. Borai, "Effect of Supplementation with Chitosan on Weight, Cardiometabolic, and Other Risk Indices in Wistar Rats Fed Normal and High-Fat/High-Cholesterol Diets Ad Libitum", Nutrition and Metabolic Insights 10:(2017) 1-8.

[51] I.Girish, and I. Harish, "a comparative study of effects of smoking on lipid profile among healthy smokers and non-smokers", Original Research Article.5:(2018) 696-699.

[52] M. Rashan, O. Dawood, H. Razzaq, and M. Hassali, "The Impact of Cigarette Smoking on Lipid Profile among Iraqi Smokers. International", Journal of Collaborative Research on Internal Medicine and Public Health,8 :(2016) 491-501

[53] N.El-Sherbeeny, M. Nader, G. Attia, and H.Ateyya, "Agmatine protects rat liver from nicotine-induced hepatic damage via antioxidative, antiapoptotic, and antifibrotic pathways", Naunyn-Schmiedeberg's Arch Pharmacol389: (2016)1341–1351.

[54] E. Ozcelika, S. Uslub, N. Erkasapc, and H. Karimib, "Protective effect of chitosan treatment against acetaminophen-induced hepatotoxicity ", The Kaohsiung Journal of Medical Sciences 30:(2014)286-290.

[55] Z.Wang, M.wang, D. Yu, Y. Zhao, H. Xu, S.Zhong ,W. Sun, Y. He, J. Niu, P. Gao, and H. li,"Therapeutic effect of chitosan on CCl4-induced hepatic fibrosis in rats", Molecular Medicine Reports 18:(2018) 3211-3218.

[56] C. Jalili, M. Salahshoor, M. Moradi, M. Ahookhash, M. Taghadosi, M. Sohrabi, "Expression Changes of Apoptotic Genes in Tissues from Mice Exposed to Nicotine", Asian Pac J Cancer Prev; 18(1):(2018) 239-244.

[57] A.El-Moslemany, "The Protective Effect of Some Cereals and Nuts on Side Effects for Nicotine on Rats", Academic Journal of Nutrition, vol. 6: (2017)14-23.

[58] W. Aboulthana, and N. Ibrahim, "A renoprotective role of chitosan against lithium-induced renal toxicity in rats"1GH. Bulletin of the National Research Centre 42(34) :(2018)-1-11.

[59] H.Ismail, "Protective effect of Resveratrol against nephrotoxicity of nicotine in male rat: Antioxidant and histopathological approaches", Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS) 3: (2017) 125-132.

[60] M. Salahshoor, S. Roshankhah, V. Motavalian, and C. Jalili, "Effect of Harmine on Nicotine-Induced Kidney Dysfunction in Male Mice",Int J Prev Med;(2019) 10:97.

[61] C.Login,A. Mureşan, C. Cătoi,S. Clichici,M. Oargă, Adriana, A. Filip, A. Nagy, F. Flaviu, Tăbăran, F., Doina, D.Daicoviciu, N. Decea, N.,and R. Moldovan, "The Protective Effect of Chitosan against Acute Oxidative Liver Injuries Induced by Carbon Tetrachloride", Bulletin UASVM, Veterinary Medicine 66(1): (2010)1843-5378.

[62] W. Xia, P. Liu, J. Zhang, and J. Chen, "Biological activities of chitosan and chitooligosaccharides", Food Hydrocolloids;25(2):(2011)170–179.

[63] D. Ngo, and S. Kim, "Antioxidant Effects of Chitin, Chitosan, and Their Derivatives: Advances in Food and Nutrition Research, (2014) 15–31.

[64] Y. Ma, Q. Huang, M. Lv, Z. Wu, Z. Xie, X. Han, and Y. Wang, Chitosan-Zn chelate increases antioxidant enzyme activity and improves immune function in weaned piglets. Biol Trace Elem Res; 158(1):(2014)45–50.

[65] N. Subhapradha, R. Saravanan, P. Ramasamy, A. A. Srinivasan, V. Shanmugam, and A.Shanmugam. "Hepatoprotective Effect of β-Chitosan from Gladius of Sepioteuthis lessoniana Against Carbon Tetrachloride-Induced Oxidative Stress in Wistar Rats", Appl Biochem Biotechnol; 172:(2014)9–20.

[66] C. Xie, X. Wu, C. Long, Q. Wang, Z. Fan, S. Li, and Y. Yin," Chitosan oligosaccharide affects antioxidant defense capacity and placental amino acids transport of sows", BMC Vet Res 12 :(2016) 243.

[67] H.Toz, andY. Değer, "The Effect of Chitosan on the Erythrocyte Antioxidant Potential of Lead Toxicity-Induced Rats", Biol Trace Elem Res 184:(2018) 114–118.

[68] F.Khatibi, A, Yaghoubi, and N. Rahbani, "Study of antioxidant enzymes, lipid peroxidation, lipid profile and immunologic factor in coronary artery disease in East Azarbijan", Int J Med Biomed Res, 1:(2012)147-52.

[69] M. Zaghloul, M. Nader, H. El-Kashefand N. Gamiel, "Beneficial effect of lipoic acid on nicotine-induced vascular endothelial damage in rabbits: Oxid Antioxid Med Sci 2(3):(2013) 175–180.

[70] H.Dhouib, M. Jallouli, M. Draief, S. El-Fazaa, and S. Bouraoui, "The Effect of Nicotine and its Interaction with Ethanol on Biochemical Parameters, Oxidative

Damage and Histological Changes in the rats",OSR Journal of Environmental Science, Toxicology and Food Technology 8(1):(2014) 72-82.

[71] M. Al Anany, A. Kamal, and K. El Saied, "Effects of curcumin and/or quercetin on nicotine-induced lung and liver toxicity in adult male albino rat", Al-azhar Assiut Medical Journal 13:(2015) 94-104.

[72] I.Oyeyipo, Y. Raji, and A.Bolarinwa," Nicotine Alters Serum Antioxidant Profile in Male Albino Rats", North American Journal of Medical Sciences 6: (2018)168-172.

[73] W. Yuan, B. Liu, C. Liu, X. Wang, M. Zhang, X. Meng, and X. Xia, "Antioxidant activity of chito-oligosaccharides on pancreatic islet cells in streptozotocin-induced diabetes in rats", World journal of gastroenterology, 15(11):(2009) 1339–1345.

[74] S. Muthumaran, A. Sudheer, V. Menon, and N. Nalini, "Protective effect of quercetin on nicotine-induced prooxidant and antioxidant imbalance and DNA damage in Wistar rats", Toxicology; 243: (2018)207–215.

[75] A.Chakraborty, S. Chowdhury, and M. Bhattacharyya, "Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes patients", Diabetes Research and Clinical Practice 93(1): (2011) 56–62.

[76] C. Jalili, H. Tabatabaei, S. Kakaberiei, S. Roshankhahand M.Salahshoor, "Protective Role of Crocin Against Nicotine-induced Damages on Male Mice Liver", Int J Prev Med.;(2015) 6:92.

[77] C. Kim, J. Choi, S. Joo, E. Bae,S. Ma, J. Lee, andS. Kim, "Nicotine-induced apoptosis in human renal proximal tubular epithelial cells", Int J Prev Med, 11, (2016) e0152591.

[78] Z. Ibrahim, M. Alkafafy, M. Ahmed, and M. Soliman, "Reno protective effect of curcumin against the combined oxidative stress of diabetes and nicotine in rats", Molecular Medicine Reports; vol. 13: (2017)3017-3026.

[79] A. Ramalingam, T. Santhanathas, A. Shaukat, and S. Zainalabidin, "Resveratrol Supplementation Protects against Nicotine-Induced Kidney Injury", Int. J. Environ. Res. Public Health 16:(2019) 4445.

الملخص العربى

التأثير التحسيني للشيتوزان على سمية النيكوتين في الجرذان المصابة بمرض السكري

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الملخص:

يعتبر الشيتوزان منتج طبيعي ويستخدم كألياف غذائية. استكشفت هذه الدراسة تأثير الشيتوزان في حالة سمية النيكوتين في ذكور الجرذان البالغة الأصحاء وذكور الجرذان المصابة بداء السكري. حيث تم تقسيم ثمانين من الجرذان البالغة والتي تزن 10g±190إلى قسمين قسم سليم وقسم مصاب بالسكري. كل قسم مقسم إلى أربع مجموعات تحتوي كل مجموعة على 10 فئران، ومجموعة ضابطة، ومجموعة تتناول الشيتوزان عن طريق الفم، ومجموعة تتناول النيكوتين ومجموعة تتناول كل من الشيتوزان والنيكوتين .لقد استغرقت التجربة 6 أسابيع. أوضحت نتائج المؤشرات الحيوية لمرض السكري أن إحداث مرض السكري أدي إلى زيادة معنوية في مستوى السكر في الدم وفي مقاومة الخلايا للأنسولين HOMA-IR وتسبب في انخفاض ملحوظ في مستوي الأنسولين في الدم ومحتوى الجليكوجين في الكبد وإنزيم الجلوكوكينيز في الكبد. كما أدى تناول النيكوتين إلى تفاقم تلك المؤشرات الحيوية. بينما لوحظ أن الشيتوزان له تأثير محسن على المؤشرات الحيوية لمرض السكري التي تسبب بها مرض السكري وحده أو مع النيكوتين. غيّر مرض السكري وتناول النيكوتين مستوي الدهون في الدم، حيث تسبب في زيادة في الدهون الثلاثيه TAGs والكوليسترول TC والدهون منخفض الكثافة LDL-C والدهون منخفض الكثافة جدا VLDL-C وعامل الخطورة RF ومؤشر التصلب AI. بينما حسن الشيتوزان COS بشكل ملحوظ مستوي الدهون في المجموعات الصحية ومرضى السكري. أدى إحداث مرض السكري إلى زيادة في نشاط إنزيمات النقل الأميني ALT وAST وخفض مستوي ألبومين، كما تسبب النيكوتين في تلف كبدي ملحوظ، بينما أدى COS إلى تحسين تأثيرات النيكوتين والسكري. تسبب مرض السكري في حدوث تغيير في وظائف الكلى. أظهرت علامات التأكسد والإنزيم المضاد للأكسدة في المجموعة المصابة بالسكري جهدا تأكسديًا في خلايا البنكرياس، مما أدى إلى انخفاض الإنزيمات المضادة للأكسدة وزيادة أكسدة الدهون. تسبب في انخفاض كبير في نشاط إنزيمSOD وزيادة مستوي MDA. بينما أظهرت نتائج تناول الشيتوزان زيادة كبيرة في نشاط إنزيم SOD في البنكرياس وانخفاض في مستوي MDA. يزيد الإجهاد التأكسدي الناجم عن تناول النيكوتين من مستوى MDA ويقلل من نشاط انزيم SOD. كما لوحظ تأثير كلا من داء السكري والنيكوتين في زيادة تفتيت الحمض النووي. بينما عمل الشيتوزان على حماية الحمض النووي من التلف. وفي نهاية الامر اوضحت تلك الدراسة تأثير الشيتوزان المضاد للسكري والمضاد للأكسدة للسمية.