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Biochemical effects of *Hydroclathrus clathratus* on alloxan -induced diabetes in rats: Role of insulin, oxidative stress and inflammation

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ABSTRACT

Background: Oxidative stress associated with insulin –dependent diabetes mellitus is a risk for inflammatory disorders that induce renal dysfunction. *Hydroclathrus clathratus* is used for its antioxidant and antiinflammatory effects. This study aims to evaluate antihyperglycemic, antihyperlipidemic, antioxidant and anti inflammatory effects of aqueous *Hydroclathrus clathratus* extract (HCE) against alloxan induced diabetes in male albino rats and explore possible effects of HCE on insulin and oxidative stress profile. Biochemical observations were further substantiated with histological examination of pancreas and kidney. **Method:** This study was carried on 30 male albino rats weighing 190 ± 10 , 90 days old, classified into 3 groups, control (NC), diabetic (DC) and treated diabetic group (HCE). Alloxan was given in a dose of 300 mg/kg body weight intraperitoneally (IP). **Result:** Alloxan produced significant increase in serum glucose, Triglyceride (TG), Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), urea and creatinine (Cr), renal reduced glutathione (GSH), renal nitrotyrosine, renal TNF- α . On other hand, alloxan produced significant decrease in insulin levels and activity of renal catalase (CAT), renal glutathione peroxidase (GSH-Px) and renal superoxide oxide dismutase (SOD). Oral HCE (300 mg/kg body weight of rats daily for 30 days) significantly ameliorated these effects. **Conclusion:** Treatment with HCE ameliorated DM and its related late consequences. Furthermore, it has antioxidant and anti-inflammatory effects. Commonly, HCE is a way to surmount the diabetic state and it has antioxidant and antiinflammatory effects. It may be a promising adjuvant to anti diabetic therapy.

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KEYWORDS

Hydroclathrus clathratus;
Diabetes mellitus;
 Lipid profile;
 Oxidative stress;
 Inflammation.

INTRODUCTION

Diabetes Mellitus (DM) is a heterogeneous disorder characterized by a progressive decline in insulin action

(insulin resistance), followed by the inability of β -cells to compensate for insulin resistance (pancreatic beta cell dysfunction^[19].

Diabetics and experimental animal models exhibit

high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation^[17].

The source of oxidative stress is a cascade of ROS leaking from the mitochondria. This process has been associated with the onset of type 1 diabetes (T1DM) via the apoptosis of pancreatic β -cells, and the onset of (T2D) via insulin resistance^[18].

Chronic and sustained high toxic levels of ROS are associated with several pathological conditions including inflammatory diseases and the complications of diabetes^[8]. Tumor necrosis factor- α (TNF- α) is an important proinflammatory cytokine involved in the pathogenesis of autoimmune T1D. Low-grade inflammation is a common feature in subjects with T2D. Heart disease, the metabolic syndrome and T2D all have in common the increased concentration of circulatory cytokines as a result of inflammation^[12].

Nitrotyrosine (N-tyr) was initially proposed to be a specific marker of increased oxidative stress related to the generation of peroxynitrite from nitric oxide in vascular endothelial cells and other tissues. Although several non-nitric oxide biosynthetic pathways for N-tyr have also been described, in all cases tyrosine nitration is believed to involve free radical chemistry reactions^[7].

DM is associated with a large number of lipid abnormalities, which in turn depend on the extent of insulin deficiency, insulin resistance, obesity, diet and the presence of concomitant primary and other secondary causes of hyperlipemia. In diabetic hyperlipemia, a series of bizarre lipoproteins and other lipids appear and interaction of this with oxidative stress and free radicals leads to enhanced lipid peroxidation in plasma, tissues and membranes, causing extensive tissue damage^[22].

Diabetic renal dysfunction whose incidence is up to 47.66% is the most common and difficult diabetic microvascular complication to treat and has become the first cause of end-stage renal disease. It is reported that about 43% of the chronic renal failure (CRF) patients on dialysis are DN, 60% case fatality of diabetes mellitus (DM) patients are DN, DM patients who died of renal failure are 17 times more than non-DM patients^[24].

Herbal remedies that stem from Egyptian traditional

medicine hold a great promise against DM. *Hydroclathrus clathratus* (C. Agardh) Howe is brown marine algae and considered to be a traditional drug and health food in Korea, Japan and China. Hot water extract of *H. clathratus* (HCE) is rich in water-soluble sulfated polysaccharides that exhibit valuable biological effects as anticancer, anti-herpetic and anti-coagulant activities^[10].

The present work was conducted to evaluate the possible hypoglycemic, hypolipidemic, antioxidant and anti-inflammatory properties of HCE on alloxan-induced type II diabetes mellitus (DM).

MATERIAL AND METHODS

Materials

(a) Experimental animals

30 White male albino rats weighting about 190 ± 10 g were used as experimental animals in the present investigation. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2 °C) and humidity ($55 \pm 5\%$) with 12 h light and 12 h dark cycle and were fed a standard diet of known composition, and water ad libitum. The chow was purchased from El-Gomhoria Company, Cairo, Egypt. They were housed for two weeks for accommodation. Our work was carried out in accordance with the guidelines of El Minia University for animal use. These animals were used for induction of *Diabetes mellitus*.

Plant material preparation of HCE

About 100 g of the fresh alga, corresponding to 10 – 12 g of dry alga material was homogenized in 500 ml hot double distilled water. The mixture was clarified by filtration using Whatman No.1 filter paper and the light brown extract resulted. The water extract of *H. clathratus* were sterilized by filtration and autoclaving, respectively.

The extract was prepared at the Department of agriculture chemistry, Faculty of Agriculture, Minia University.

Experimental design and animal grouping

Experimental *Diabetes* was induced in 20 rats by a dose of 150 mg/kg intraperitoneally (IP)^[31]. Above

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200 mg/dl blood glucose level were considered diabetic rats. These DM animals were divided into 2 groups, one served as untreated DM group, the other as treated DM group, which was given HCE as 300 mg/kg body weight of rats daily for 30 days. Control rats were injected with same volume of vehicle (saline buffer). At the end of the experiment, all groups were bled by vein puncture, fasting blood samples were centrifuged, sera were kept at -80°C right the time of analysis, meanwhile, kidney and brain were collected, blotted between 2 filter papers, frozen directly into liquid Nitrogen, then kept at -80°C till tissue biochemical investigations.

Methods

(a) Biochemical investigations in blood and tissue

Biochemical estimations in blood and serum insulin: Fasting blood glucose (FBG) concentration of all the three experimental groups was determined by glucometer during different phases of the experiment by withdrawing blood from the tail vein. Serum insulin was assayed in the Radioactive Isotopes Unit, Central Department of Scientific Analysis and Test, National Research Center (Dokki, Giza) by radioimmunoassay kits of DPC (Diagnostic Products Corporation, Los Angeles, USA). For estimating serum lipid profile, serum was isolated from the blood collected by cardiac puncture under mild ether anesthesia from overnight fasted rats on day 30th of MCD treatment and serum total cholesterol (TC)^[3] and triglyceride (TG)^[6] using diagnostic kits (Erba Mannheim Cholesterol kit, Transasia Bio-Medicals Ltd., Daman). Results were expressed in mg/dl. On other hand, serum urea and creatinine^[30] were also measured.

For determination of tissue biochemical parameters, rats were sacrificed by decapitation and incisions were

immediately done for separation of the kidney. The isolated kidney was quickly weighted and dissected into pieces, homogenized in volumes of ice cold de-ionized water to yield 20% W/V homogenate using ice cold Teflon homogenizer (Potter Elvehjem type). Also, renal reduced glutathione (GSH)^[5], nitrotyrosine^[16], TNF- α ^[19a] and activity of renal (CAT)^[26], GSH-Px^[25] (SOD)^[11].

(b) Histopathological examination of pancreas

Some pancreas were cleaned and fixed in 10% neutral buffered formalin solution. After dehydration in graded ethanol solutions and in toluene, they were embedded in paraffin. Sections of 3–5 μm thickness were stained with hematoxylin and eosin (H.E.) for histopathological examination.

(c) Histopathological examination of kidney

Some Kidney were cleaned and fixed in 10% neutral buffered formalin solution. After dehydration in graded ethanol solutions and in toluene, they were embedded in paraffin. Sections of 3–5 μm thickness were stained with hematoxylin and eosin (H.E.) for histopathological examination.

Statistical analysis

Statistical analysis was carried out using Graph Pad Instat software (version 3, ISS-Rome, Italy). Groups of data were compared with ANOVA, followed by Tukey-Kramer (TK) multiple comparisons post-test. Values of $P < 0.05$ were regarded as significant. Data were expressed as mean \pm standard error (SEM).

RESULTS

Serum glucose levels were significantly increased,

TABLE 1 : Effect of HCE on studied blood parameters in diabetic rats, compared to normal control (Values are expressed in M \pm SE, N= 10 for each group)

Parameter	NC	DC	HCE
Glucose (mg/dl)	112.3 \pm 9.8	465 \pm 41.8 *** ^a	241 \pm 18.9 ^b
Insulin (ng/ml)	1.18 \pm 0.07	0.48 \pm 0.07 *** ^a	1.06 \pm 0.06 ^b
Triglycerides (mg/dl)	74.2 \pm 6.8	178.2 \pm 10.4 *** ^a	103.7 \pm 8.3 ^b
T Cholesterol (mg/dl)	87.2 \pm 8.4	163.2 \pm 7.9 *** ^a	96.4 \pm 6.3 ^b
H.D.L-C (mg/dl)	63.2 \pm 5.7	39.3 \pm 4.5 *** ^a	50.2 \pm 3.9 ^b
L.D.L-C (mg/dl)	13.5 \pm 7.3	53.2 \pm 4.8 *** ^a	26.3 \pm 11 ^b

a***Significantly different from control at $P < 0.001$. b***Significantly different from DM at $P < 0.001$

TABLE 2 : Effect of HCE on studied renal parameters in diabetic rats, compared to normal control (Values are expressed in M±SE, N= 10 for each group)

Parameter	NC	DC	HCE
Renal MDA nM MDA/mg protein	110.23±14.36	974.23±28.12*** ^a	679.45±45.2 ^b
Renal GSH mg/gm tissue	15.23±1.06	9.36±0.89*** ^a	26.26±3.24 ^b
Renal Nitrotyrosine (nM)	124.36±8.13	253.68±4.43*** ^a	199.35±7.04 ^b
Renal NO (nmol/mg protein)	1.87±0.17	4.24±0.3*** ^a	3.1±1.7 ^b
Renal CAT μ moles H ₂ O ₂ decomposed/min/mg protein	274.39±12.36	163.26±10.36*** ^a	301.36±19.68 ^b
Renal GSH-Px μg GSH consumed/min/mg protein	752.36±73.13	375.62±34.11*** ^a	643.32±45.69 ^b
Renal SOD Units/min/mg protein	350.28±24.18	274.29±21.48*** ^a	315.49±20.36 ^b
renal TNF-α (pg/gm)	151±8.5	614±52.9*** ^a	423.1±29.97 ^b
renal caspase-3(U/mg protein)	0.87±0.06	1.9±0.19*** ^a	0.91±0.08 ^b
Urea (mg/dl)	25.41±2.14	66.14±2.14*** ^a	40.41±1.69 ^b
Creatinine (mg/dl)	0.65±0.07	1.31±0.01*** ^a	1.05±0.02 ^b

a***Significantly different from control at P < 0.001. b***Significantly different from DM at P < 0.001

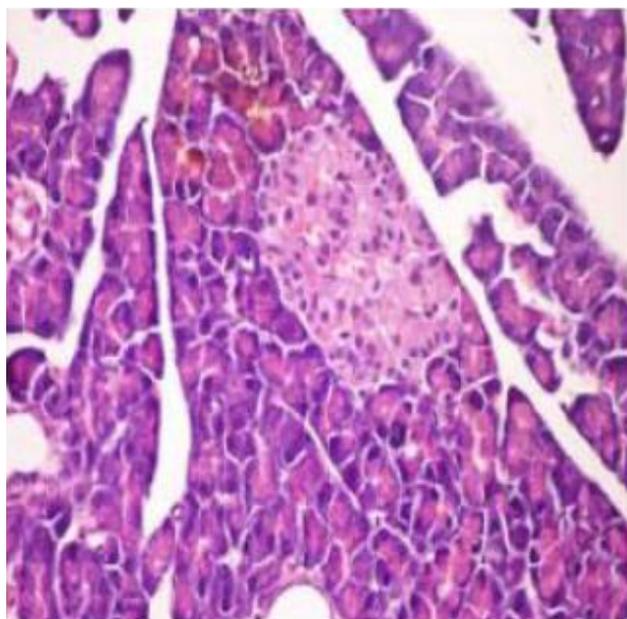


Figure 1 : Pancreatic tissue of normal male albino rats. The pancreas is subdivided by septa into pancreatic lobules. The exocrine portion of the pancreas consists of pancreatic acini while endocrine portion consists of islets of Langerhans (H&E x 400)

but insulin levels were decreased in alloxan diabetic rats compared to control, and treatment with HCE significantly reduced glucose but non significantly elevated insulin in relation to diabetic rats. Both triglycerides, total cholesterol and LDL-C levels were significantly increased, while HDL-C was significantly decreased by induction of diabetes, these figures were significantly ameliorated after HCE treatment (TABLE 1). Activity of renal (CAT), (GSH-Px) and (SOD) were significantly

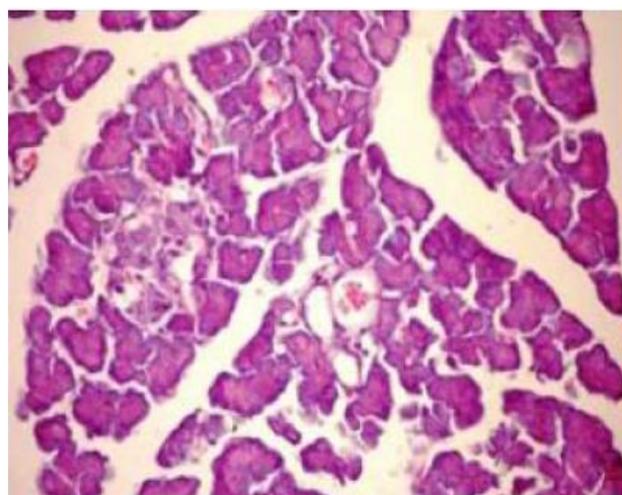


Figure 2 : Pancreatic tissue of diabetic rats. Normal architecture of the islets is disrupted islets of Langerhans exhibited hydrophobic cells, necrotic cells, vacuolizations and irregular hyperchromic nuclei (H&E x 400)

decreased in the alloxan group compared to control, treatment with HCE significantly ameliorated these changes (TABLE 2).

Also, renal MDA, nitrotyrosine, NO, TNF-α, urea and Creatinine contents were significantly increased, in the alloxan group compared to control, treatment with HCE significantly ameliorated these changes (TABLE 2).

On other hand, Levels of renal GSH were significantly decreased in the alloxan group compared to control, treatment with HCE significantly changed these parameters (TABLE 2) In contrast, Activity of renal caspase was significantly increased in the alloxan

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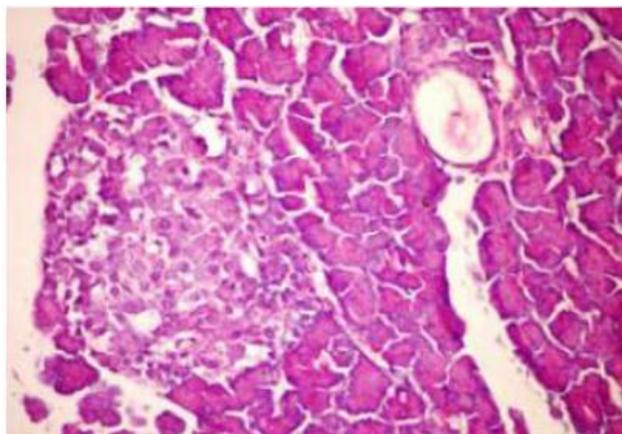


Figure 3 : Pancreatic tissue of diabetic rats treated with *HCE* (orally 300 mg/kg body weight of rats daily for 30 consecutive days) showing hypertrophy and vacuolations of B cells of islets of langerhans (H&E x 400)

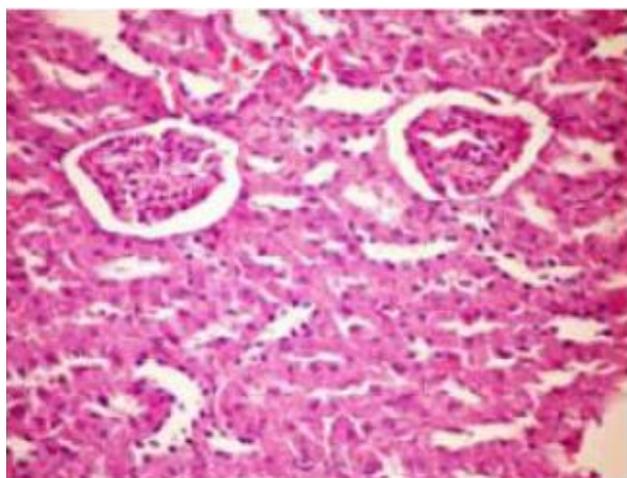


Figure 4 : Kidney of normal rat showing normal histological structure of renal parenchyma (H&E×400)

group compared to control, treatment with *HCE* significantly decreased the activity of renal caspase (TABLE 2).

DISCUSSION

Alloxan is toxic glucose analogues that preferentially accumulate in pancreatic beta cells *via* the Glucose transporter -2 (GLUT2). In the presence of intracellular thiols, especially glutathione; alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ul-

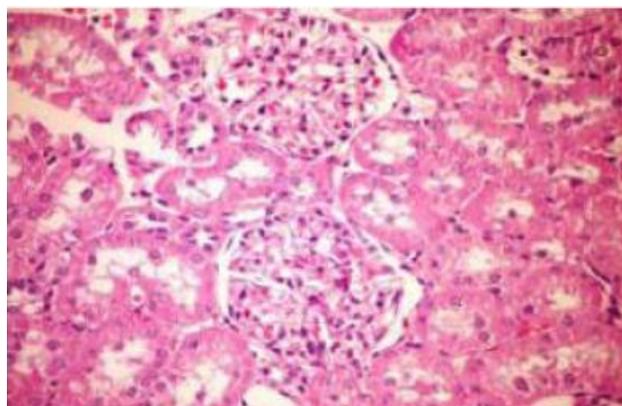


Figure 5 : Kidney of control diabetic rat showing vacuolization of endothelial lining glomerular tufts and epithelial lining renal tubules (H&E×400)

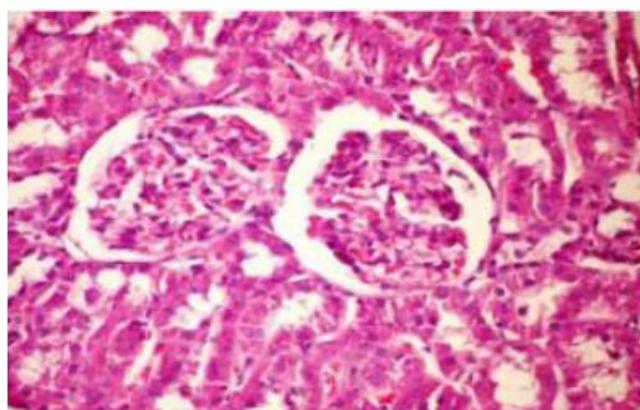


Figure 6 : Kidney of diabetic rat treated by *HCE* (orally 300 mg/kg body weight of rats daily for 30 consecutive days) showing slight vacuolization of endothelial lining glomerular tufts (H&E×400)

imately responsible for the death of the beta cells, which have a particularly low antioxidative defence capacity^[27].

The present investigation indicated that a single dose of alloxan (150 mg/kg) intraperitoneally to adult male albino rats (190±10 g) was suitable to induce histological changes of the islets of Langerhans characterized appearance, hypoinsulinemia and hyperglycemic state. The present dose as well as the observed histopathological and biochemical manifestations agree with the literature of^[13].

A gradual loss of β -cells due to apoptosis significantly hinders insulin production and inhibits cell viability. During apoptosis, cells shrink; chromatin condenses; DNA is cleaved into pieces at inter nucleosomal regions. A proactive way to increase β -cell viability is to decrease apoptosis level in order to retain the cell popu-

lation and increase insulin production^[23].

Exposure of islets to alloxan shows significantly increased formation of peroxynitrite, NO and ROS with markedly elevated lipid peroxidation and reduced cell viability. Islets exposed to alloxan also show significantly increased mitochondrial membrane potential. Apparently, alloxan causes severe oxidative and cytotoxic stress to islets that is likely to compromise their insulin releasing capacity^[14].

Oral administration of HCE causes significant decrease in levels of blood glucose although the significant increase in level of serum insulin in agreement with^[15].

Huang et al., 2005^[9b] explained that sulphated polysaccharides from marine algae are known to be important free-radical scavengers and antioxidants for the prevention of pancreatic oxidative damage, which is an important contributor in DM. Furthermore,^[33] revealed that Fucoindans, major component in either HCE can decrease apoptosis and activity of caspase-3 at lysosome-cathepsin D level.

Antidiabetic effect of HCE was further examined by histological observations made on the pancreatic tissue of HCE treated rats that revealed hypertrophy and vacuolations of β - cells of islets of langerhans.

In our study, elevated levels of serum TC, TG, LDL and VLDL-cholesterol and decreased HDL cholesterol concentration in alloxan-induced diabetic rats are in accordance with^[32] that represents the characteristic features of diabetic dyslipidemia are that attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/resistance which in turn resulting in excess fatty acid accumulation in the liver, which is converted to triglycerides.

Moreover,^[4] revealed that DM increase the generation of NADPH oxidase-derived reactive oxygen species and induce apoptosis of glomerular epithelial cells (podocytes) and loss of podocytes contributes to progression of kidney disease.

Oral administration of either HCE causes significant decrease in the serum levels triglycerides, total cholesterol and LDL-cholesterol in contrast to significant elevation in HDL-cholesterol and body weight gain in accordance with^[10].

Park et al, 2011^[21] revealed that sulphated polysaccharides, major component in HCE enhance the

negative charges of cell surface so as to effect the aggradation of cholesterol in blood, as a result decreasing the cholesterol in serum.

Alloxan led to a significant increase in serum urea, serum creatinine, renal MDA, renal nitrotyrosine levels and activity of renal caspase-3. On other hand, Alloxan causes significant decrease in renal GSH content and significant decrease in activity of renal SOD, CAT and GPx. A similar effect was recorded by^[1,29].

Santamaria et al., 2008^[27] revealed that enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea and creatinine. Furthermore, Alloxan increased the productions of reactive oxygen species, enhanced lipid peroxidation and protein carbonylation in association with decreased intracellular antioxidant defense in the kidney tissue. In addition, hyperglycemia enhanced the levels of proinflammatory cytokines (TNF- α).

Cao et al, 2011^[2] suggests that development of diabetic renal dysfunction may due to activation of endoplasmic reticulum stress that can mediate progressive endothelial damage through growth and migration of vascular smooth muscle and inflammatory cells, alteration of extracellular matrix, apoptosis of endothelial cells, over-expression of inflammatory cytokines as TNF- α .

Moreover, Eid et al^[4] revealed that DM increase the generation of NADPH oxidase-derived reactive oxygen species and induce apoptosis of glomerular epithelial cells (podocytes) and loss of podocytes contributes to progression of kidney disease.

Also, Navarro and Mora-Fernandez^[20] revealed that TNF α -pathway has a broad range of inflammatory and apoptotic properties and dysregulation of these processes may contribute to injury of the diabetic kidney as TNF α -pathway directly increases glomerular vasoconstriction and albumin permeability. Exposure of the kidney to TNF α increases mRNA expression of TNF receptors in renal tubulointerstitium and triggers cell death.

Oral administration of HCE causes significant decrease in level of serum urea, serum creatinine, renal MDA and renal TNF- α But, HCE causes significant enhancement in level of renal GSH and the activity of renal CAT, renal GSH-Px and renal SOD.

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On other hand, HCE causes significant decrease in activity of renal caspase-3 in accordance with^[10]. Sulphated poly saccharides in HCE alleviate cathepsin D and caspase-3 activation, improve cell survival, and ameliorate epithelial mesenchymal transition and renal fibrosis in diabetic rats^[33].

Fucoidan oligosaccharides show good protective effects on renovascular diabetic rats and one of the mechanisms underlying the antihypertensive effects might be that they can inhibit the production of plasma angiotensin II. On other hand, Anti inflammatory effect of HCE may due to sulphated polysaccharides, fucoidan which is a potent modulator of connective tissue proteolysis that occurs during inflammatory diseases where, continuous supply of inflammatory cells and exacerbated production of inflammatory cytokines is present^[28].

Reno- protective effect of HCE was further evidenced by histological observations made on the renal tissue of HCE treated rats that revealed slight vacuolization of endothelial lining glomerular tufts.

CONCLUSIONS

Treatment with HCE improved associated metabolic consequences to Type I DM, showing hypoglycemic, insulin sensitization, and antioxidant and hypolipidemic actions. The results suggest HCE as a beneficial adjuvant for the treatment of type I diabetes mellitus and possibly as a protector against long term nephropathy.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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REFERENCES

[1] V.B.Brito, J.B.da Rocha, G.O.Puntel, S.C.da Luz, N.B.Barbosa, N.R.de Carvalho, V.Folmer; Inhibition of δ -aminolevulinatase is not closely

related to the development of hyperglycemia in alloxan-induced diabetic mice (2011).

- [2] Y.P.Cao, Y.M.Hao, Q.J.Liu, J.Wang, H.Li, H.J.Duan; threlationship between endoplasmic reticulum stress and its particular apoptosisway caspase-12 and apoptosis in renal cortex of diabetic rats. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*, **27(2)**, 236-3 (2011).
- [3] E.De Bakey Michael, M.Antonio, J.R.Gotto; "Atherosclerosis" in the New Living Heart, Holbrook. MA:Adams Media Corporation (1997).
- [4] A.A.Eid, B.M.Ford, K.Block, B.S.Kasinath, Y.Gorin, G.Ghosh-Choudhury, J.L.Barnes, H.E.Abboud; AMP-activated protein kinase (AMPK) negatively regulates Nox4-dependent activation of p53 and epithelial cell apoptosis in diabetes, *J.Biol.Chem.* **26**, **285(48)**, 37503-12 (2010).
- [5] G.L.Ellman; Tissue sulfhydryl groups, *Archives of Biochemistry*, **82**, 70–77 (1959).
- [6] P.Fossati, L.Prencipe; Serum triglycerides determined colourmetrically with an enzyme that produces hydrogen peroxide, *Clin.Chem.*, **28(1)**, 2077-2080 (1982).
- [7] A.Gumieniczek, M.Wilk; Nitrosative stress and glutathione redox system in four different tissues of alloxan-induced hyperglycemic animals, *Toxicol Mech Methods*, **19(4)**, 302-7 (2009).
- [8] E.Hatanaka, P.T.Monteagudo, M.S.Marrocos, A.Campa; Neutrophils and monocytes as potentially important sources of proinflammatory cytokines in diabetes, *Clin Exp Immunol*, **146**, 443–7 (2006).
- [9] (a) Hesham.A.El-Beshbishy, Ola.M.Tork, Mohamed.F.El-Bab, Mohamed.A.Autifi; Antioxidant and antiapoptotic effects of green tea polyphenols against azathioprine-induced liver injury in rats, *Pathophysiology*, **18**, 125–135 (2011); (b) Z.X.Huang, X.T.Mei, D.H.Xu, J.Y.Lv; Protective effects of polysacchride of *Spirulina platensis* and *Sargassum thunbeergii* on vascular of alloxan induced diabetic rats, *Zhongguo Zhong Yao Za Zhi*, **30(3)**, 211-5 (2005).
- [10] R.G.Jayasooriya, G.Y.Kim, W.J.Kim, Y.H.Choi; Methanol extract of *Hydroclathrus clathratus* suppresses matrix metalloproteinase-9 in T24 bladder carcinoma cells by suppressing the NF- κ B and MAPK pathways, *Oncol Rep*; **27(2)**, 541-6 (2012).
- [11] P.Kakkar, B.Das, P.N.Viswanathan; A modified spectrophotometric assay of superoxide dismutase, *Ind.J.Biochem.Biophys*, **21**, 130-32 (1984).
- [12] A.M.Kampoli, D.Tousoulis, A.Briasoulis, G.Latsios,

- N.Papageorgiou, C.Stefanadis; Potential pathogenic inflammatory mechanisms of endothelial dysfunction induced by type 2 diabetes mellitus, *Curr Pharm Des*, **17(37)**, 4147-58 (2011).
- [13] V.P.Lebedev, S.V.Bilichenko, N.E.Ordyan, S.G.Pivina, S.P.Nechiporenko, A.A.Puzyrev, E.A.Mikheeva, K.K.Kubacheva; Transcranial electrostimulation activates reparative regeneration and the insulin-producing function of pancreatic B-cells in alloxan diabetes in rats, *Neurosci Behav Physiol.*, **37(4)**, 341-7 (2007).
- [14] E.Lee, G.R.Ryu, S.H.Ko, Y.B.Ahn, K.H.Yoon, H.Ha, K.H.Song; Antioxidant treatment may protect pancreatic beta cells through the attenuation of islet fibrosis in an animal model of type 2 diabetes, *Biochem.Biophys.Res.Commun.* **22**, **414(2)**, 397-402 (2011).
- [15] H.Liu, L.Gu; Phlorotannins from brown algae (*Fucus vesiculosus*) inhibited the formation of advanced glycation endproducts by scavenging reactive, *J.Agric.Food Chem.* **8**, carbonyls, **60(5)**, 1326-34 (2012).
- [16] Löwik, papaoulas, Van; Effect of interleukin-17 on nitric oxide production and osteoclastic bone resorption: is there dependency on nuclear factor-kappaB and receptor activator of nuclear factor kappaB (RANK)/RANK ligand signaling? *Bone*, **28(4)**, 378-86 (2001).
- [17] K.M.Mellor, R.H.Ritchie, Delbridge; Reactive oxygen species and insulin-resistant cardiomyopathy, *Clin Exp Pharmacol Physiol*, **37(2)**, 222-8 (2010).
- [18] F.A.Matough, S.B.Budin, Z.A. Hamid, N.Alwahabi, J.Mohamed; The role of oxidative stress and antioxidants in diabetic complications, *Sultan Qaboos Univ Med J*, **12(1)**, 5-18 (2012).
- [19] M.A.Nagy, S.A.Mohamed; Antidiabetic effect of *Cloem Droserifolia* (*Cloemaceae*), *American Journal of Biochemistry*, **4(4)**, (68-75) (2014).
- [20] J.F.Navarro, C.Mora-Fernandez; The role of TNF-alpha in diabetic nephropathy: Pathogenic and therapeutic implications, *Cytokine Growth Factor Rev*, **17**, 441-450 (2006).
- [21] M.K.Park, U.Jung, C.Roh; Fucoïdan from marine brown algae inhibits lipid accumulation, *Drugs*, **9(8)**, 1359-67 (2011).
- [22] Y.Peña, J.E.Fernández-Britto, J.Bacallao, J.F.Batista, M.L.de León; Lipid levels as predictors of silent myocardial ischemia in a type 2 diabetic population in havana, *MEDICC Rev*, **14(1)**, 18-24 (2012).
- [23] S.I.Rabbani, K.Devi, S.Khanam; Role of Pioglitazone with Metformin or Glimepiride on Oxidative Stress-induced Nuclear Damage and Reproductive Toxicity in Diabetic Rats, *Malays J Med Sci.* **17(1)**, 3-11 (2010).
- [24] M.H.Rosner, C.Ronco, M.D.Okusa; The role of inflammation in the cardio-renal syndrome: a focus on cytokines and inflammatory mediators, *Semin Nephrol*, **32(1)**, 70-8 (2012).
- [25] J.T.Rotruck, A.L.Pope, H.F.Ganther, A.B.Swanson; Biochemical role as a component of glutathione peroxidase, *Science*, **179**, 588-90 Selenium (1973).
- [26] A.K.Sinha; Colometric assay of catalase, *Anal Biochem*, **47**, 389-94 (1972).
- [27] B.Santamaría, A.B.Sanz, A.C.Ucero, M.D.Sánchez-Niño, A.Benito-Martín, A.Reyero, M.Ruiz Ortega, R.Selgas, A.Ortiz; Apoptosis: from advances in PD to therapeutic targets in DM, *Nefrologia* **28 Suppl**, **6**, 23-6 (2008).
- [28] K.Senni, F.Gueniche, A.F.Bertaud, S.I.Tchen, F.Fioretti, S.C.Jouault; Fucoïdan a sulfated polysaccharide from brown algae is a potent modulator of connective tissue proteolysis, *Archives of Biochemistry and Biophysics*, **445**, 56-64 (2006).
- [29] C.Soto, J.Pérez, V.García, E.Uría, M.Vadillo, L.Raya; Effect of silymarin on kidneys of rats suffering from alloxan-induced diabetes mellitus, *Phytomedicine.* **1**, **17(14)**, 1090-4 (2010).
- [30] L.Thomas; *Clinical Laboratory Diagnostics*. 1st Edition Frankfurt: TH - Books Verlagsgesellschaft, 374-7 (1998).
- [31] Thippeswamy, A.Core, R.V.Kullarni; Antihyperglycemic and antihyperlipidemic activity of plectranthus amboinicus on normal and alloxan-induced diabetic rats, *Indian.J.Pharm.Sci.*, **73(2)**, 139-45 (2011).
- [32] U.C.Yadav, K.Moorthy, N.Z.Baquer; Combined treatment of sodium orthovanadate and Momordica charantia fruit extract prevents alterations in lipid profile and lipogenic enzymes in alloxan diabetic rats, *Mol Cell Biochem.*, **268(1-2)**, 111-20 (2005).
- [33] G.Zhang, P.Li, Y.Li, X.Liu, Y.Chen, X.Weng, Q.Yang, X. Zhu; Fucoïdan by inhibiting cathepsin D activities alleviates PC12apoptosis induced by hydrogen peroxide, *Zhongguo Zhong Yao Za Zhi*, **36(8)**, 1083-6 (2011).