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Biochemical and histopathological analysis of *Cystoseira myrica* aqueous extract on alloxan induced diabetic rats

M.A.Nagy[1]*, K.A.Amin[2]

[1]Chemistry Department, Faculty of Science, Beni-Suef University, (EGYPT)

[2]Biochemistry Department Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, (EGYPT)

E-mail : nagy_bio@yahoo.com

ABSTRACT

Objective: To investigate antidiabetic, hypolipidemic histopathological analysis of *Cystoseira myrica* aqueous (CME) extract in alloxan induced diabetic rat by administering oral dose (400 mg/kg body weight /day). **Methods**: Optimal cutoff level of each of the four plasma glucose values of oral glucose tolerance test in alloxan diabetic rat was done. Other parameters as liver profile, renal profile, cardiac profile and total lipid levels were determined in normal and alloxan induced diabetic rats after oral administration of the extract for 30 days. Histopathological changes in diabetic rat organs (pancreas, liver, kidney and heart) were also observed after extract treatment. **Results**: Daily oral administration CME (400 mg/kg body weight) and glimebride (10 microg/g body wt) showed beneficial effects on blood glucose level ($P<0.001$) as well as improving kidney, liver functions and hyperlipedemia due to diabetes. The extract treatment also showed enhanced serum insulin level and body weight of diabetic rats as compared to diabetic control group. Furthermore, the extract has a favorable effect on the histopathological changes of the pancreas and kidney in alloxan induced diabetes. **Conclusion**: *Cystoseira myrica* posses antidiabetic property as well as improve body weight, liver profile, renal profile and total lipid levels. CME has also favorable effect to inhibit the histopathological changes of the pancreas and kidney in alloxan induced diabetes.

KEYWORDS

Biochemical Profiles;
Cystoseira myrica.

INTRODUCTION

Diabetes mellitus (DM) is a very commonly occurring metabolic disorder characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins. DM occurs due to absolute or relative deficiency of insulin or insulin resistance[12].

This metabolic disorder affects approximately 4% of the population worldwide and is expected to be increased by 5.4% in 2025. Because DM is associated with oxidative stresss, it alters the cellular microenvironment in many different types of tissues causing myriad untoward effects, collectively referred to as 'diabetic complications'. Two cellular processes affected by diabetes are inflammation and apoptosis[33].

Glimepiride is a medium-to-long acting sulfonylurea

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anti-diabetic drug. sometimes classified as the first third-generation sulfonylurea and sometimes classified as second-generation. Glimepiride acts as an insulin secretagogue. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors[37].

Synthetic drugs usually come with considerable side effects, such as hypoglycemia, drug-resistance, dropsy, and weight gain In recent years due to the adverse effects of synthetic hypoglycemic drugs, interests in alternate therapeutic approach have become very popular Nowadays, herbal drugs are gaining popularity in the treatment of diabetes and its complications due to their efficacy, low incidence of side effects and low Cost.

The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads. Some of the plants which are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the WHO expert committee on diabetes recommends that this area warrant further attention[36].

Brown marine algae to be rich sources of antioxidant compounds with potential free radical scavenging activity that may be useful in prevention and treatment of various diseases caused by oxidative damage. Fucoidans, polysaccharides containing substantial percentages of L-fucose and sulfate ester groups, are constituents of brown algae that have numerous other biological properties such antioxidant, anti-inflammatory immuno-modulatory and apoptosis-inducing activities[35].

Cystoseira myrica (S. G. Gmelin) C. Agardh (Gulf of Suez) is brown marine algae that can be classified as one of the more advanced species (phytochemically) of the Cystoseira genus based on the complexity of the terpenes produced. Hot water extract of C. *myrica* (CME) is rich in bioactive metabolites derived from algae are as sulfated polysaccharides that have antioxidant activity[24].

MATERIALS AND METHODS

Glimepiride

The drug was purchased from (Delta Pharma for Pharmaceuticals, Egypt). Oral dose: Oral administration of (10 microg/g body wt) daily in accordance with[18].

Plant material

Mature whole *Cystoseira myrica* were collected from El- Zafrana, Gulf of Suez. by Mr.khalid el yamani, Department of botany, faculty of science, Beni suef university, Egypt and authenticated as *Cystoseira myrica* by Dr.H.Ezzat, Department of Pharmacognacy, Faculty of pharmacy, Minia university, Egypt.

Extract preparation

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 C. myrica were sterilized by filtration and autoclaving, respectively.

Chemicals

Alloxan was purchased from Loba chemie Pvt. Ltd. Mumbai, India. Total cholesterol (TC), serum high-density lipoprotein (HDL), serum Creatinine (SC), serum urea (SU), alanine transaminase (ALT), serum aspartate transaminase (AST) and triglyceride (TG) standard kits were obtained from Erba diagnostics Mannheim Gambh, Germany. Blood glucose level was measured using Elegance glucose meter (CT-X10) of Convergent Technologies, Germany. All reagents used in study were analytical grade.

Animals

40White 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±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.*

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TABLE 1 : Effect of CME on optimal cutoff level of each of the four plasma glucose values of oral glucose tolerance test in alloxan induced diabetic rats (A-D)(n=10).

	Optimal cutoff level			
Group	Fasting glucose level	1. h glucose level	2. h glucose level	3. h glucose level
Normal (N)	69.12±2.3	112.3±9.8	89.3±7.2	74.9±6.8
Diabetic Control (DC)	142±12.3 ***[a]	465±41.8 ***[a]	435±30.25 ***[a]	412±38.5 ***[a]
Diabetic +Glimepride (G)	176±16.2 [a]	484±40.28 [a]	401±29.8 [b]	314±27.1 [c]
Diabetic+ CME (CME)	131±102 [b]	430±36.9 [b]	374±32.2 [b]	355±24.1 [b]

*** Values significantly different compared to normal P***‹0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p‹0.05) based on one –way ANOVA with Tukey's post –hoc test.**

Induction of diabetes

Rats were made diabetic by a single intraperitoneal njection of alloxan monohydrate (Loba Chemie, Bombay; 150 mg/kg i.p.) in sterile saline. Twelve days after Alloxan injection, rats with blood glucose level of >200 mg/dL were separated and used for the study. Blood glucose levels were measured using blood glucose test strips with elegance glucometer (Frankenberg, Germany) at weekly intervals till the end of study (i.e. 3 weeks). Blood glucose estimation was done on 0, 1, 2 and 3 hours after administration of extract orally at the 30 th day of starting experiment.

Experimental design

Overnight fasted rats were divided into four groups and for each group 10 animals and treated orally once a day for 30 days as follows:

- Group I: Normal healthy control: given only vehicle (Tween)
- Group II: Diabetic control: given only vehicle (Tween 80, 5%80, 1% v/v) v/v)
- Group III: Diabetic rats given Glimepiride (10 microg/g body wt.)
- Group IV: Diabetic rats given CME 400 mg/ kg body weight /day

Biochemical parameters

Optimal cutoff level of each of the four plasma glucose values of oral glucose tolerance test in alloxan diabetic was measured with elegance glucometer (Frankenberg, Germany) at hour intervals i.e. 0, 1, 2 and 3 hours after daily administration of extract orally. After blood glucose estimation on day 30, whole blood was collected by cardiac puncture under mild ether anesthesia from rats. Serum cholesterol, triglycerides, creatinine, urea, and HDL cholesterol levels were also evaluated in normal and alloxan induces diabetic rats. Serum alanine transaminase (ALT) and serum aspartate transaminase (AST), LDH, CK-NAC[21] and CK-MB[38] were measured by autoanalyser (Erba Chem 7, Mannheim, Germany) using Erba diagnostic kits. Serum insulin levels were determined using insulin ELISA ki.

TABLE 2 : Effect of CME on serum insulin level in alloxan induced diabetic rats (A-D)(n=10)

Group	Serum insulin levels (ng/ml)
	After 30 days of treatment
Normal (N)	1.18±0.07
Diabetic control (DC)	0.48±0.07***[a]
Diabetic + Glimepride (+G)	0.62±0.04 [a]
Diabetic+ CME (CME)	1.12±0.07 [b]

*** Values significantly different compared to normal P***‹0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p‹0.05) based on one –way ANOVA with Tukey's post –hoc test.**

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 ± standard error (SEM).

RESULTS

Antidiabetic activity

Single dose alloxan monohydrate (150 mg/kg) significantly ($P<0.01$) increases the blood glucose as shown in TABLE 1. After the daily oral administration with CME, for 30 days, significant decreased ($P<0.01$) in the blood glucose levels was observed in the diabetic

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TABLE 3 : Effect of CME on body weight, triglycerides (T.G) and total cholesterol (T.C) in alloxan induced diabetic rats.

Group	Body weight gain	T.G	T.C
	gm	mg/dl	mg/dl
Normal (N)	37±3.21	74.2±6.8	87.2±8.4
Diabetic Control (DC)	-27±3.4^{***a}	178.2±10.4^{***a}	163.2±7.9^{***a}
Diabetic + Glimepride (G)	-21±2.6^{a}	120.3±8.6^{b}	102.3±7.4^{b}
Diabetic+ CME (CME)	+80±6.3^{b}	135.5±9.4^{b}	115.6±7.1^{b}

*** Values significantly different compared to normal P***‹0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p‹0.05) based on one –way ANOVA with Tukey's post –hoc test.**

TABLE 4 : Effect of CME on HDL cholesterol and LDL cholesterol in alloxan induced diabetic rats

Group	HDL.C	LDL.C
	mg/dl	mg/dl
Normal (N)	63.2±5.7	13.5±7.3
Diabetic Control (DC)	39.3±4.5^{***a}	53.2±4.8^{***a}
Diabetic + Glimepride (G)	45.2±3.4^{b}	35.1±3.3^{b}
Diabetic+ CME (CME)	43.2±4.1^{b}	35.2±3.0^{b}

*** Values significantly different compared to normal P***‹0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p‹0.05) based on one –way ANOVA with Tukey's post –hoc test.**

rats (TABLE 1. The reduced insulin level in diabetic rats was also significantly improved by treatment of CME. At the end of experiment (30thday) blood glucose level was (355±24.1) mg/dL of the groups treated with CME (TABLE 2).

Effect on body weight of rats

In diabetic rats, continuous reduction in body weight was observed as shown in TABLE 2. CME treatment significantly (P<0.05) improved the body weight of diabetic rats.

Effect on lipid profile

In diabetic rats, there was a significant increase of serum total cholesterol, triglycerides (TABLE 3) and significant decrease in HDL cholesterol in compared to that of normal control. The standard drugs as well as CME used in the experimental study significantly decreased (P<0.05) the levels of cholesterol and triglycerides whereas HDL cholesterol significantly increased (TABLE 4).

Effect on liver functions

The effect of CME on liver functions is represented in the TABLE 5. ALT, AST were significantly elevated in alloxan induced diabetes. The rats treated with CME showed non significant (P<0.01) reduction in the elevated levels of liver enzymes (transaminase) as shown in TABLE 5.

Effect on renal functions

Kidney function markers like urea and creatinine were elevated in the alloxan induced diabetic rats when compared with the normal rats. CME reduced both the levels in dose dependent manner (TABLE 6).

Effect on cardiac functions

The effect of HCE on cardiac functions is represented in the TABLE 5. LDH, CK-NAC and CK-MB were significantly elevated in alloxan induced diabetes. The rats treated with HCE showed non significant (P<0.01) reduction in the elevated levels of LDH, CK-NAC and CK-MB as shown in TABLE 7.

Histology of pancreas

Histology of pancreas (Figure 1) showed normal acini, and normal cellular in the islets of langerhans in the pancreas of normal control (1.a). In diabetic animals treated extensive damage to islets of langerhans and reduced dimensions of islets were observed in diabetic rats (1.b). On other hand, CME treatment showing hypertrophy and vacuolations of B cells of islets of langerhans treatment (1.d).

Histology of liver

Photomicrographs of liver (Figure 2) showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein (2.a). In case of group II diabetic rats, the normal lobular structure was preserved. The central vein was prominent and prominently congested. Focal areas of hemorrhage were also seen. Fatty

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TABLE 5 : Effect of CME on liver parameters in normal and diabetic rats

Group	sALT	sAST
	U/l	U/l
Normal (N)	19.51±0.84	23.17±0.75
Diabetic Control (DC)	62.39±0.87***[a]	84.29±0.94***[a]
Diabetic + Glimepride (G)	59.18±0.91[a]	70.06±0.89[a]
Diabetic+ CME (CME)	53.19±0.74[a]	55.25±0.71[a]

*** Values significantly different compared to normal P***‹0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p‹0.05) based on one –way ANOVA with Tukey's post –hoc test.**

TABLE 6 : Effect of CME on kidney parameters in normal and diabetic rats (n=10)

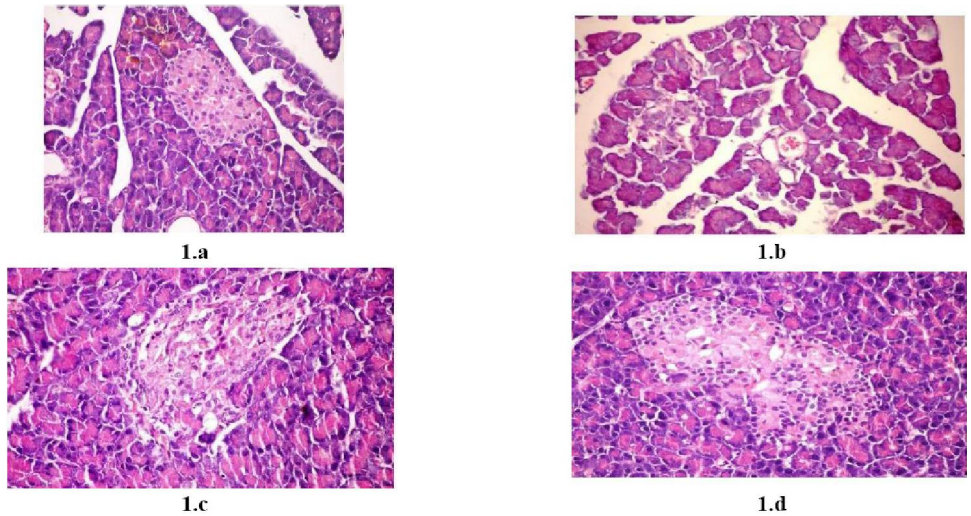
Group	Urea	Creatinine
	(mg/dl)	(mg/dl)
Normal (N)	25.41±2.14	0.65±0.07
Diabetic Control (DC)	66.14±2.14***[a]	1.31±0.01***[a]
Diabetic + Glimepride (G)	29.26±2.65[c]	0.98±0.03[b]
Diabetic+ CME (CME)	38.29±2.87[b]	1.02±0.01[b]

*** Values significantly different compared to normal P***‹0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p‹0.05) based on one –way ANOVA with Tukey's post –hoc test.**

TABLE 7 : Effect of CME on cardiac parameters in normal and diabetic rats

Group	L.D.H	CK.NAC	CK.MB
	(u/I)	(u/I)	(u/I)
Normal (N)	243.18±23.36	264.15±23.36	141.38±12.36
Diabetic Control (DC)	532.26±25.12***[a]	574.29±31.24***[a]	274.15±13.80***[a]
Diabetic + Glimepride (DC +G)	448.65±36.26[b]	416.8±41.28[b]	193.57±13.69[b]
Diabetic+CME (DC +CME)	524.28±27.88[a]	565.18±36.36[a]	263.57±16.59[a]

*** Values significantly different compared to normal P***‹0.001; * Values are expressed as means ± SE. Means not sharing common letter are significantly different (p‹0.05) based on one –way ANOVA with Tukey's post –hoc test.**



1.a 1.b 1.c 1.d

Figure 1 : Effect of CME on pancreas of alloxan induced diabetic rats

change was evident.

The portal tracts appeared normal (2.b). In group IV [diabetic rats + CME 400 mg/kg], In contrast, (2.d) shows kupffer cells activation and congestion of central

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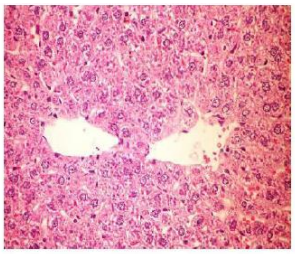
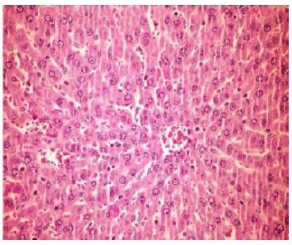


2.a



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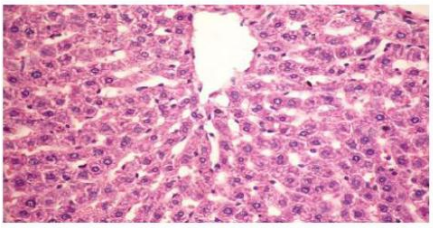

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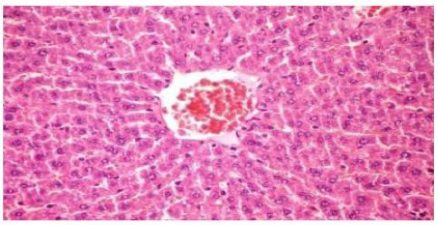
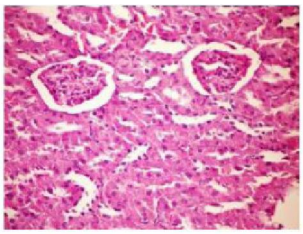
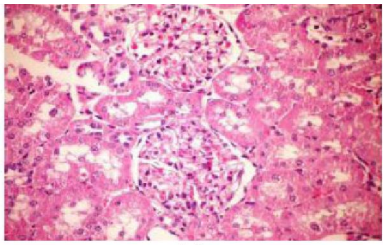


2.d

Figure 2 : Effect of CME on liver of alloxan induced diabetic rats



3.a



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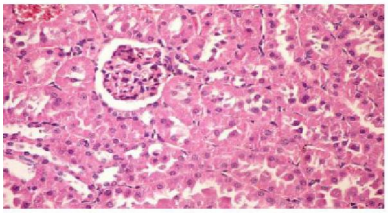

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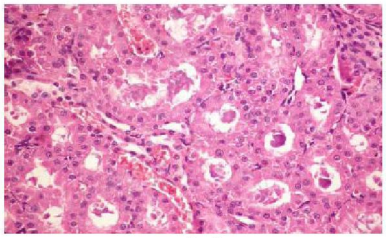
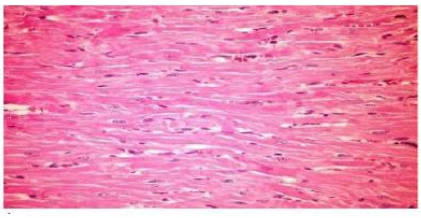
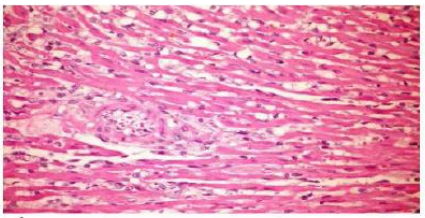


3.d

Figure 3 : Effect of CME on kidney of alloxan induced diabetic rats



4.a



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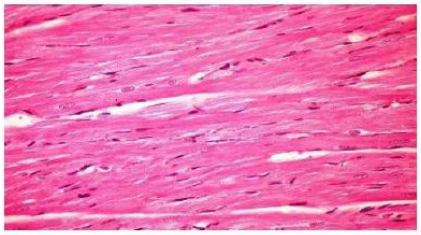
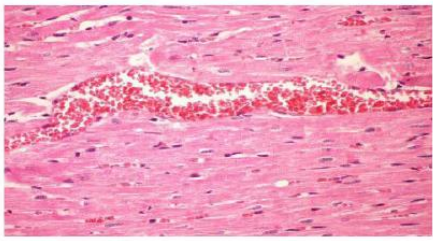


4.c



4.d

Figure 4 : Efffect of CME on heart of alloxan induced diabetic rats

vein (2.d).

Histology of kidney

Histology of kidney (Figure 3) in normal animals showed normal structure (3.a). In diabetic rats, mild thickening of the basement membrane of the arterioles of glomeruli along with mild change of density of mesangial mesangium were observed. No other significant changes were seen (3.b). After CME 400 mg/kg treatment, (3.d) shows epithelial protein cast in the lumen of renal tubules.

Histology of heart

Histology of heart (Figure 4) in normal animals showed normal structure (4.a). In diabetic rats, vacuolization of sarcoplasm of myocytes was observed. No other significant changes were seen (4.b). After CME 400 mg/kg treatment, congestion of myocardial blood vessel was shown (4.d).

DISCUSSION

Glucose is the key physiological regulator of insulin secretion; indeed, short-term exposure of â-cells to increasing glucose concentrations induces proliferation in a concentration-dependent manner. In addition to its effect on β-cell turnover, hyperglycemia also impairs β-cell secretary function. This glucotoxic effect is evident before apoptosis leads to a significant decrease in β-cell mass[6].

The present investigation indicated that a single dose of alloxan **(**150 mg/kg) intraperitoneally to adult male albino rats (210-220g) 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[15].

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 population and increase insulin production.

Oral administration of glimepiride causes significant decrease in levels of blood glucose in accordance with who revealed that glimepiride exerts its insulin-releasing effect mainly by inhibiting ATP-sensitive potassium channels. In the pancreatic β-cell this action induces depolarization of the cell membrane, allowing an influx of calcium in the cell. This in turn induces insulin release into the blood.

[26]revealed that the increase in the number of β-cells in the islets of Langerhan's in glimepiride-treated diabetic rats in comparison to alloxan induced diabetic rats can be attributed to the fact that glimepiride affect the activation of the redox sensitive transcription factor NF (Kappa) β in alloxan induced diabetic rats. Although the mechanism of β-cell neoformation is not clear but there is strong evidence that islet stem cells may exist in the pancreatic duct and that these ductal epithelial cells may be switched into a proliferative /regenerative phase leading to nesideoblastosis (neogenesis of islets).

Antidiabetic effect of glimepiride was further examined by histological observations made on the pancreatic tissue of glimepride treated rats that show vacuolization and necrosis of some β cells of islets of Langerhans.

Oral administration of either CME causes significant decrease in levels of blood glucose although the significant increase in level of serum insulin in agreement with Liu and Gu[17].

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.

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

The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL-cholesterol concentration and increased concentration of small dense LDL-cholesterol particles. The lipid changes associated with *diabetes mellitus* are attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/ resistance. This results in excess fatty acid accumulation in the liver, which is converted to triglycerides. The impaired ability of insulin to inhibit free fatty-acid release leads to elevated

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hepatic VLDL-cholesterol production. The increased VLDL-cholesterol and triglyceride levels decrease the level of HDL-cholesterol and increase the concentration of small dense LDL-cholesterol particles by activation of lipoprotein lipase and lecithin acyl-cholesterol transferase[23].

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[39].

On other hand, Induction of diabetes by alloxan resulted in loss of body weight in the diabetic control rats in accordance with that may due to catabolic effect on protein metabolism by retarding protein synthesis and stimulating protein degradation.

Oral administration of glimepiride causes significant decrease in the serum levels of triglycerides, total cholesterol and LDL-cholesterol in contrast to significant elevation in HDI.-cholesterol and body weight in accordance with[8].

Motoyama et al[22] revealed that glimepiride improved HDL-c level via improvement of plasma adiponectin level as adiponectin could increase HDL-c levels directly via increased lipoprotein lipase and decreased hepatic lipase activity. On other hand, the antilipidemic action of glimepiride may reside in their ability to stimulate insulin secretion and action.

Oral administration of either CME causes significant decrease in the serum levels triglycerides, total cholesterol and LDL-cholesterol in contrast to significant elevation in HDI.-cholesterol and body weight gain in accordance with[13].

Park et al[25] revealed that sulphated poly saccharides, major component in either CME 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.

The significant increase in body weight gain in CME treated diabetic rats may due to anabolic effect on protein metabolism by stimulating protein synthesis and retarding protein degradation.

Renal dysfunction is a slowly progressive process that is postulated to be accelerated by intervening diseases, such as diabetes, due in part to the addition of excessive stress and inflammation[9].

DM causes a disturbance in the uptake of glucose, as well as glucose metabolism. The liver plays an important role in the maintenance of blood glucose levels by regulating its metabolism[32].

The present investigation indicated that, a single dose of alloxan **(**150 mg/kg) intraperitoneally to adult male albino rats (210-220g) was suitable to induce histological changes in the liver of alloxan induced diabetic rats with characterized appearance, enlarged and swollen hepatocytes[10].

Alloxan causes significant increase in activity of sAST and sALT, in accordance with that is directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of DM and are also responsible for the increased gluconeogenesis and ketogenesis[2].

DM induces the growth of HSCs via MAP kinase pathways, which are activated by ROS produced by the NADPH oxidase system under the regulation of protein kinase C. On other hand, hepatic oxidative stress induces proinflammatory cytokines, such as TNF-α, transforming growth factor-β (TGF-β), interleukin-1β, and interleukin-6, which are critical for HSC activation and perpetuation[34].

Alloxan led to a significant increase in serum urea and serum creatinine A similar effect was recorded by[3]. [30]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[9].

Cao et al[5] 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, overexpression of inflammatory cytokines.

Oral administration of glimepiride causes significant decrease in level of seum urea and creatinine in accordance with[29].

In the present study the improvement in blood urea,

serum creatinine and subsequent amelioration of histomorphological changes in kidneys of glimepiride treated rats can be attributed to the recovery of renal function which is explained by the regenerative capability of the renal tubules as good metabolic control is beneficial in slowing the progression of renal dysfunction in diabetes[20].

Glimepiride could ameliorate the glomerular and tubular lesions that characterize diabetic renal dysfunction and subsequently recover renal morphology and function. Reno- protective effect glimepiride of was further evidenced by histological observations made on the renal tissue of glimepiride treated rats that revealed normal structure of renal parenchyma

Oral administration of CME causes significant decrease in level of serum urea, serum creatinine in accordance with[11].

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 angiorensin II. On other hand, Anti inflammatory effect of CME 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[31].

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

Several studies have suggested that carbohydrate and lipid metabolic abnormalities, such as hyperglycemia and hyperlipidemia, may contribute to the development of cardiac dysfunction in DM. A significant reduction in myocardial glucose supply and utilization has been observed in isolated diabetic cardiomyocytes, which could be the primary injury in the pathogenesis of this specific heart muscle disease Therefore, it is necessary to increase glucose utilization or the rate of glucose transport in the diabetic heart[14].

Alloxan led to a significant increase in activity of myocardial L.D.H, C.K-NAC, C.K-MB in accordance with[28]. [4]revealed that reduced muscle mitochondrial content function with DM would lower the total cellular ATP yield, which would result most notably in increased mitochondrial volume, and increased glycolytic enzymes necessitating increased activity of creatine kinase, as this enzyme is responsible for rapidly transferring high-energy phosphate groups from the site of production to the site of use.

Oral administration of glimepiride causes significant decrease in activity of L.D.H, CK.NAC and CK.MB in accordance with[7] who revealed that glimepiride, improved endothelial function despite similar glycemic control. The improvement in endothelial function was mainly due to a reduction in insulin resistance.

CONCLUSION

Although additional studies are needed, it could be suggested that CME have Antidiabetic, Antihyperlipidemic mechanisms against Pancreas, liver, kidney injury induced by alloxan. The signaling mechanisms associated with protection against the liver damage and oxidative stress status induced by alloxan via intake CME still need merit further investigations.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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