ORIGINAL ARTICLES

Effect of Moderate Ethanol Administration on Biochemical Indices in Streptozotocin-diabetic Wistar Rats

OA Adaramoye[1], GK Oloyede[2]

ABSTRACT

Objective: *This study was designed to evaluate the effect of moderate ethanol administration on the biochemical indices in streptozotocin (STZ)-diabetic rats.*

Methods: *Twenty-four male Wistar rats were divided into four groups of six animals each. Groups one and two contained non-diabetic normal rats and normal rats treated with ethanol, respectively. Group three was untreated STZ-diabetic rats and group four was made up of ethanol-treated STZ-diabetic rats. Diabetes was induced by a single intraperitoneal injection of STZ (35 mg/kg), while ethanol (10%$^{v}/_{v}$) was given at a dose 2 g/kg thrice per week for three weeks. After the last dose of ethanol and an overnight fasting, rats were sacrificed by cervical dislocation. Blood was collected by syringe from the heart into plain centrifuge tubes.*

Results: *Moderate ethanol administration to STZ-diabetic rats caused a significant ($p < 0.05$) increase in relative weight of liver relative to normal. Ethanol intake in STZ-diabetic rats produced an insignificant ($p > 0.05$) effect on the levels of fasting blood glucose (FBG) and HbA_{1c} relative to the untreated-diabetic group. Moderately, ethanol administration to STZ-diabetic rats produced a marked and significant ($p < 0.05$) increase in the levels of serum total cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol and the activities of alanine aminotransferase relative to untreated diabetic rats. Ethanol-treated diabetic rats had significantly ($p < 0.05$) lower high-density lipoprotein (HDL)-cholesterol levels, while the activities of lactate dehydrogenase and α-amylase were insignificantly ($p > 0.05$) affected. There were no significant ($p > 0.05$) differences in all the biochemical indices in normal rats relative to ethanol-treated normal rats.*

Conclusions: *Moderate ethanol administration did not affect FBG and HbA_{1c}, but altered the lipid profile of STZ-diabetic rats. Moderate ethanol intake may further increase the risk of complications in diabetes.*

Keywords: Biochemical indices, diabetes, ethanol, lipid profile, streptozotocin

Efecto de la Administración Moderada de Etanol Sobre los Índices Bioquímicos en Ratas Wistar Diabéticas por Estreptozotocina

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RESUMEN

Objetivo: *Este estudio se diseñó con el propósito de evaluar el efecto del uso de etanol moderado sobre los índices bioquímicos en ratas Wistar diabéticas por estreptozotocina (STZ).*

Métodos: *Veinticuatro ratas Wistar machos fueron divididas en cuatro grupos de seis animales cada uno. Dos de los grupos tenían ratas normales no diabéticas y ratas normales tratadas con etanol, res-*

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pectivamente. El tercer grupo estaba formado por ratas diabéticas por STZ no tratadas, y el cuarto por ratas diabéticas por STZ tratadas con etanol. La diabetes fue inducida mediante una inyección intraperitoneal de STZ (35 mg/kg), mientras que el etanol (10%$^{v}/_{v}$) fue administrado en dosis de 2 g/kg tres veces por semana durante tres semanas. Tras la última dosis de etanol y un ayuno de una noche, las ratas fueron sacrificadas mediante dislocación cervical. La sangre fue recogida del corazón con jeringuillas e introducida en tubos para centrífuga sin graduación.

Resultados: *La administración moderada de etanol a ratas diabéticas por STZ, causó un aumento significativo (*$p < 0.05$*) en el peso relativo del hígado con relación al normal. La ingestión de etanol en ratas diabéticas por STZ tuvo un efecto insignificante (*$p > 0.05$*) en los niveles de glucosa en sangre en ayuno (GSA) y HbA_{1C} en relación con grupos diabéticos no tratados. En medida moderada, la administración de etanol a ratas diabéticas por STZ produjo un aumento marcado y significativo (*$p < 0.05$*) en los niveles de colesterol total en suero, triglicéridos, el colesterol asociado con las lipoproteínas de baja densidad, o colesterol LDL, y la actividad de la aminotransferasa alanina en relación con las ratas diabéticas no tratadas. Las ratas diabéticas tratadas con etanol tuvieron niveles significativamente disminuidos de colesterol asociado con las lipoproteínas de alta densidad, o colesterol HDL, en tanto que la actividad del lactato deshidrogenasa y la α-amilasa no fue afectada significativamente (*$p > 0.05$*). No hubo diferencias significativas (*$p > 0.05$*) en todos los índices bioquímicos en las ratas normales con respecto a las ratas normales tratadas con etanol.*

Conclusiones: *El suministro moderado de etanol no afectó el GSA ni el HbA_{1C}, pero alteró el perfil lípido de las ratas diabéticas por STZ. La ingestión moderada de etanol puede aumentar a un más el riesgo de las complicaciones de la diabetes.*

Palabras claves: Indices bioquímicos, diabetes, etanol, perfil lípido, estreptozotocina

West Indian Med J 2012; 61 (1): 4

INTRODUCTION

Individuals with uncontrolled hypertension, heart failure, hypertriglyceridaemia, liver disease, pancreatitis, deep religious belief or a strong family history of allergy to alcohol are often advised to abstain from alcohol consumption. The list also includes those who are pregnant and those who are not of legal age (1). Epidemiological studies have shown that male healthcare professionals who consumed alcohol moderately had reduced risk of angina pectoris (2), myocardial infarction (3) and stroke (4). In both men and women, moderate use of alcohol was associated with protection against congestive heart failure (5). The inverse association between alcohol consumption and cardiovascular disease was strongest in men with high plasma LDL cholesterol levels (6).

Although still under debate, recent reviews have shown that moderate alcohol consumption may be associated with a decreased incidence of diabetes (7). There are reports that moderate alcohol intake may reduce plasma glucose and insulin levels in diabetes (8). In addition, light to moderate drinkers with diabetes have also been reported to show a decreased risk for coronary heart disease (9). Likewise, a randomized crossover trial in postmenopausal women, linked moderate alcohol consumption to decrease in fasting insulin and triglyceride concentrations and increased insulin sensitivity (10). Howard *et al* (11) observed that moderate intake of alcohol by diabetic subjects does not acutely aggravate glycaemia and may produce a modest decrease in plasma glucose. From the aforementioned, there is no information on the specific effects of moderate alcohol consumption on glucose metabolism and biochemical indices in individuals with diabetes. This observation was supported by Bantle *et al* (8) who stated that the effects of alcohol in patients with diabetes have not been clearly defined. The objectives of the present study were to evaluate if moderately administered alcohol (10%$^{v}/_{v}$ ethanol at a dose of 2 g/kg body weight thrice a week for 3 weeks) to STZ-diabetic rats would be able to lower fasting blood glucose, HbA_{1c} and offer beneficial effects on biochemical indices in the serum of the animals.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Sigma Chemical Co, St Louis, Missouri, USA. Serum biochemical analysis was done using diagnostic kits from Randox, Bayer and Boehringer Mannheim. Other chemicals were of analytical grade and the purest quality available.

Animals

Male Wistar rats, 220–230 g were used for this study. The rats were 10–12 weeks of age at the time of this study. They were bred and housed in the Central Animal House, Faculty of Pharmacy, Tehran University of Medical Sciences, University of Tehran, Iran, where one of the authors (OAA) was a visiting scientist under TWAS-UNESCO Associateship programme. The animal house was well ventilated with a 12-hour light-dark cycle. They were fed on normal laboratory chow and allowed free access to water for two weeks before the commencement and during the period of the experiment. Handling

of animals and other protocols conform to the guidelines of the National Institutes of Health (NIH) [NIH publication 85–23, 1985]. The study was approved by the Animal Ethics Committee of the University of Tehran, Iran.

Study design

Rats were fasted overnight and made hyperglycaemic by a single intraperitoneal injection of STZ dissolved in 0.05M of citrate buffer (pH 4.3), at a dose of 35 mg/kg body weight (12). The blood glucose levels of these rats were estimated 72 hours after STZ administration and moderately diabetic rats having blood glucose level above 250 mg/dL were selected for this study. Twenty-four rats were randomly divided into four groups of six animals each. Group one contained non-diabetic rats (normal), group two consisted of normal rats that received ethanol, group three was untreated STZ-diabetic rats and group four was ethanol-treated STZ-diabetic rats. Ethanol ($10\%^{v}/_{v}$) was administered by oral gavage at a dose of 2 g/kg body weight (13) thrice a week for three consecutive weeks. After the last dose of ethanol, rats were fasted overnight and sacrificed by cervical dislocation. Visceral organs were obtained by dissection and immediately weighed, while blood was collected by syringe from the heart of the animals into plain centrifuge tubes.

Preparation of serum

Blood samples were allowed to stand for one hour in an air-conditioned room (temperature of about 18–20^{o}C) and then centrifuged at 3000 g for 15 minutes in an MSC bench centrifuge to obtain serum. The clear supernatant (serum) was used for the estimation of urea, creatinine, enzymes, lipid profile and other parameters.

Biochemical assays

Protein determination: Protein contents in serum were determined according to the method of Lowry *et al* (14) using bovine serum albumin as a standard.

Alanine and aspartate aminotransferases (ALT and AST) determination: Serum ALT and AST activities were assayed using the methods of Mohun and Cook (15) and Reitman and Frankel (16).

Glucose and glycosylated haemoglobin (HbA_{1c}) determination: Glucose and HbA_{1c} levels were determined by the methods of Sharma *et al* (17) and Hirokawa *et al* (18), respectively.

Determination of α-amylase and lactate dehydrogenase (LDH) activities: Serum α-amylase and LDH activities were determined by the methods of Gella *et al* (19) and Zimmerman and Weinstein (20), respectively.

Determination of total bilirubin: Serum total bilirubin levels were assayed by the method of Rutkowski and Debaare (21).

Alkaline phosphatase (ALP) determination: The estimation of alkaline phosphatase (ALP) activity was based on the method of Williamson (22).

Creatinine and urea determination: Serum creatinine and urea levels were estimated by the methods of Jaffe (23) and Talke and Schubert (24), respectively.

Determination of triglyceride and cholesterol levels: Serum triglyceride and cholesterol levels were assayed using commercial diagnostic kits (Boehringer Mannheim).

Determination of HDL-cholesterol: Lipoproteins – very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL)-cholesterol were precipitated by addition of phosphotungstic acid and magnesium chloride to serum. After centrifugation, the clear supernatant, which contained the high-density lipoprotein (HDL)-cholesterol, was assayed with Boehringer Mannheim diagnostic kit.

LDL-cholesterol determination: The LDL-cholesterol was calculated using the formula of Friedewald *et al* (25).

Determination of gamma glutamyl transferase (GGT) activities: The activities of GGT were determined according to the method of Fossati *et al* (26).

Statistical analysis: All values were expressed as the mean ± SD of six animals per group. Data were analysed using one-way ANOVA followed by post-hoc Duncan multiple range test for analysis of biochemical data using SPSS (10.0). Values were considered statistically significant at $p < 0.05$.

RESULTS

The effect of ethanol on body weight, FBG and HbA_{1c} of STZ-diabetic rats

In Fig. 1, Tables 1 and 2, the final body weight of untreated and ethanol-treated STZ-diabetic rats decreased significantly

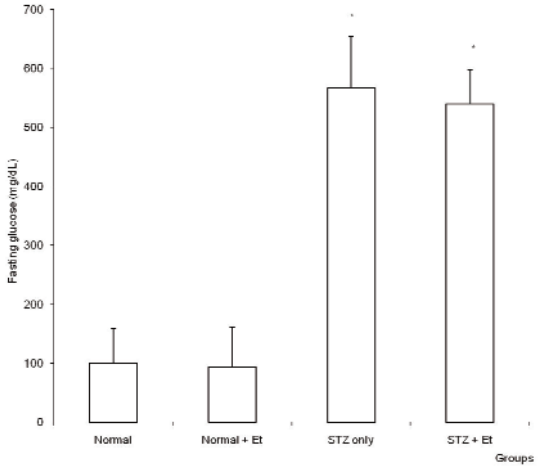

Fig 1: Effect of ethanol administration on the levels of fasting glucose of streptozotocin (STZ)-diabetic rats.

*Significantly different from normal and normal + Et ($p < 0.05$); Et = Ethanol

Table 1: Effect of ethanol administration on the bodyweight and relative weight of organs of streptozotocin-diabetic rats

Treatment	Bodyweight (g)			Weight (g)			Relative weight (% bodyweight)		
	Initial	Final	Change	Liver	Kidney	Heart	Liver	Kidney	Heart
Normal	225.1 ± 5.3	241.3 ± 6.2	16.2 ± 5.6	6.6 ± 0.5	1.3 ± 0.1	0.8 ± 0.1	2.7 ± 0.3	0.5 ± 0.1	0.3 ± 0.05
Normal + Et	220.0 ± 6.9	233.7 ± 7.8	13.7 ± 6.2	6.8 ± 0.8	1.2 ± 0.1	0.7 ± 0.1	2.9 ± 0.4	0.5 ± 0.2	0.3 ± 0.06
STZ only	226.3 ± 4.7	178.0 ± 9.1*	-48.3 ± 8.3	6.2 ± 0.8	1.2 ± 0.2	0.8 ± 0.1	3.5 ± 0.5*	0.6 ± 0.1	0.4 ± 0.05
STZ +Et	221.5 ± 6.1	162.8 ± 7.5*	-58.7 ± 6.6	8.3 ± 0.6**	1.2 ± 0.1	0.7 ± 0.1	5.1 ± 0.3**	0.7 ± 0.1	0.4 ± 0.04

Values are means ± SD of six rats per group
* Significantly different from normal and normal + Et ($p < 0.05$)
** Significantly different from normal, normal + Et and STZ only ($p < 0.05$)
STZ = Streptozotocin, Et = Ethanol

Table 2: Effect of ethanol administration on biochemical indices in streptozotocin-diabetic rats

Treatment	Protein (mg/dL)	SERUM LDH (IU/L)	Urea (mmol/L)	Red cell HbA_{1c} (%)
Normal	1.82 ± 0.46	579.8 ± 31.2	17.2 ± 2.8	4.2 ± 0.28
Normal + Et	1.68 ± 0.51	615.3 ± 58.4	19.6 ± 3.7	4.8 ± 0.33
STZ only	1.63 ± 0.38	1137.2 ± 51.3*	27.5 ± 4.4*	8.3 ± 0.36*
STZ + Et	1.75 ± 0.40	1105.6 ± 66.9*	25.1 ± 3.7*	8.4 ± 0.29*

Values are means ± SD of six rats per group
* Significantly different from normal and normal + Et ($p < 0.05$)
STZ = Streptozotocin, Et = Ethanol, HbA_{1c} = Glycosylated haemoglobin, LDH = Lactate dehydrogenase

($p < 0.05$) relative to normal. The relative weight of the liver in ethanol treated diabetic rats were significantly ($p < 0.05$) higher than that of the untreated diabetic group. Furthermore, FBG and HbA_{1c} levels of ethanol-treated and untreated diabetic animals were significantly ($p < 0.05$) elevated relative to normal. Moderate ethanol intake did not alter significantly ($p > 0.05$) the FBG and HbA_{1c} in the diabetic animals relative to untreated diabetic group. The body weight gain, FBG and HbA_{1c} levels were statistically similar ($p > 0.05$) in normal rats relative to ethanol-treated normal rats.

Effect of ethanol on the activities of serum enzymes in STZ-diabetic rats

In ethanol-treated diabetic rats, a significant ($p < 0.05$) increase in the activities of ALT was observed relative to the untreated diabetic group (Fig. 4). The activities of ALT were increased by 89% in the ethanol-treated diabetic group. Although, the activities of α-amylase and LDH were significantly ($p < 0.05$) elevated in STZ-diabetic rats (Table 2 and Fig. 6), ethanol intake produced insignificant ($p > 0.05$) differences in these parameters between treated and untreated diabetic rats. There were no significant ($p > 0.05$) differences in the activities of GGT, AST and ALP in the diabetic groups relative to normal (Figs. 3, 4 and 6). Moderate ethanol intake did not significantly ($p > 0.05$) alter the activities of ALT, AST, GGT and ALP in normal rats relative to untreated normal rats.

Effect of ethanol on the levels of bilirubin, urea, creatinine and lipids in STZ-diabetic rats

Ethanol-treated diabetic rats had significantly higher ($p < 0.05$) values of serum total cholesterol, triglyceride and LDL-cholesterol, and a lower value of HDL-cholesterol relative to the untreated diabetic group (Figs. 2 and 3). Ethanol intake produced an insignificant ($p > 0.05$) effect on the levels of serum urea between treated and untreated STZ-diabetic rats (Table 2). Serum creatinine and bilirubin did not significantly ($p > 0.05$) differ in normal rats relative to other groups (Fig. 5). Also, the serum lipid profiles of normal and ethanol-treated normal rats were statistically similar. The levels of serum cholesterol, triglycerides and LDL-cholesterol were significantly

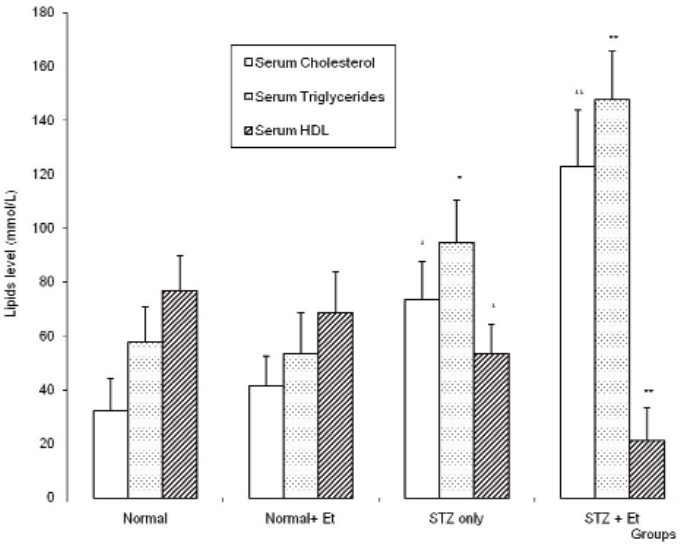

Fig 2: Effect of ethanol administration on the levels of serum cholesterol, triglyceride and HDL-cholesterol of streptozotocin (STZ)-diabetic rats.
*Significantly different from normal and normal + Et ($p < 0.05$); ** Significantly different from normal, normal + Et and STZ-only; Et = Ethanol

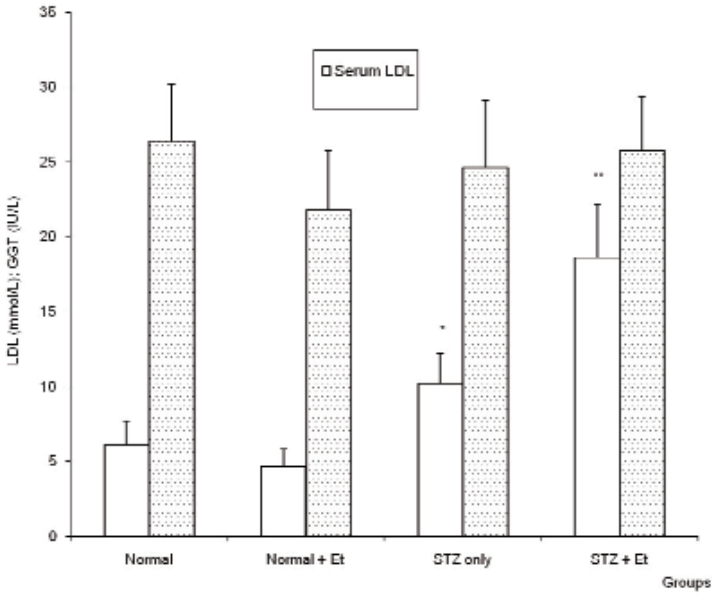

Fig 3: Effect of ethanol administration on the levels of serum LDL-cholesterol and gamma glutamyl transferase (GGT) in streptozotocin (STZ)-diabetic rats.

*Significantly different from normal and normal + Et ($p < 0.05$); ** Significantly different from normal, normal + Et and STZ-only ($p < 0.05$); Et = Ethanol

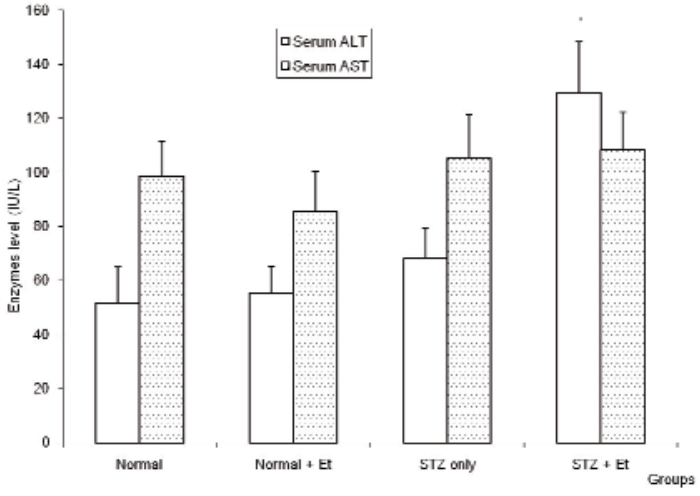

Fig 4: Effect of ethanol administration on the levels of serum alanine and aspartate aminotransferases (ALT and AST) in streptozotocin (STZ)-diabetic rats.

*Significantly different from normal, normal + Et and STZ-only ($p < 0.05$); Et = Ethanol

($p < 0.05$) elevated in untreated STZ-diabetic rats relative to normal, while HDL-cholesterol level was significantly ($p < 0.05$) lowered (Figs. 2 and 3). Furthermore, these lipid profiles differed significantly ($p < 0.05$) between ethanol-treated and untreated STZ-diabetic rats.

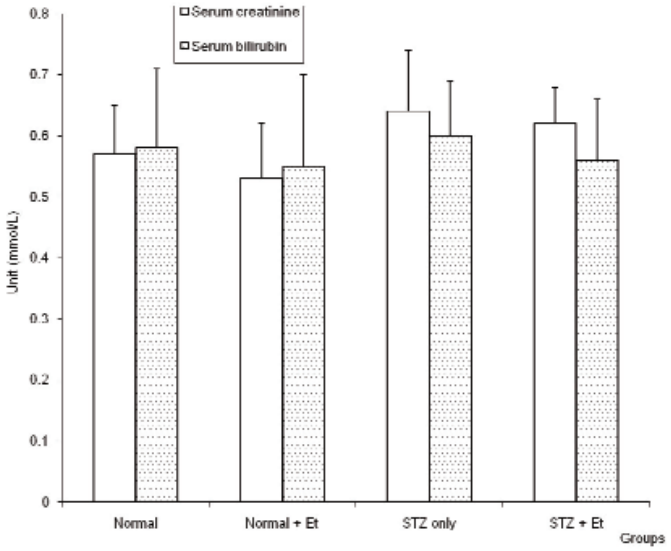

Fig 5: Effect of ethanol administration on the levels of serum creatinine and total bilirubin in streptozotocin (STZ)-diabetic rats.

Et = Ethanol

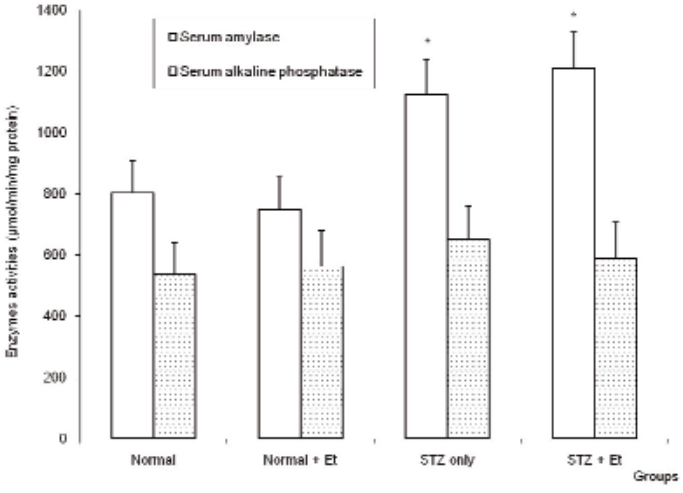

Fig 6: Effect of ethanol administration on the activities of serum alpha amylase and alkaline phosphatase of streptozotocin (STZ) – diabetic rats.

*Significantly different from normal and normal + Et ($p < 0.05$); Et = Ethanol

DISCUSSION

This study did not confirm any significant effect of moderate alcohol administration on fasting blood glucose (FBG) or glycaemic control index (HbA_{1c}) in the STZ-diabetic rats. These findings are consistent with the results of Howard *et al* (11), which state that moderate alcohol does not aggravate glycaemia in diabetic subjects. Although alcohol has been shown to inhibit hepatic gluconeogenesis in subjects with Type 2 diabetes mellitus (DM), it does so without decreasing hepatic

glucose output (27) or, presumably, such decrease in gluconeogenesis may be compensated for by increased glycogenolysis in the animals and thus explain why glycaemic conditions were not adversely affected by moderate alcohol intake. Type 2 diabetes is characterized by the coexistence of abnormal lipids, endothelial dysfunction and insulin resistance and these abnormalities are considered key factors in the development of cardiovascular complications (28). Our data showed that moderate alcohol consumption caused a marked decline in serum HDL-cholesterol and increase in total cholesterol, triglyceride and LDL-cholesterol in the diabetic rats. These observations sharply disagree with the findings of Bantle *et al* (8), who concluded that chronic wine consumption did not affect plasma HDL-cholesterol or other plasma lipid fractions in subjects with Type 2 DM. The differences in results may be due to the sources of alcohol, type of DM, subjects used and duration of the studies. While the present study used 10%$^{v}/_{v}$ absolute ethanol, the former used red wine which contains, in addition to ethanol, polyphenols such as catechin, epicatechin, procyanidins, quercetin, resveratrol, myricetin, *etc*, with strong antioxidants properties (29). Interestingly, ethanol-treated normal rats had similar lipid profiles with untreated normal rats, thus confirming that the moderate ethanol administration to STZ-diabetic rats caused the elevation of the lipid parameters except HDL-cholesterol which actually decreased. It should be noted that ethanol is a powerful indicator of hyperlipidaemia in both animals and humans, and the most common lipid abnormalities in alcoholism are hypercholesterolaemia and hypertriglyceridaemia (30), which was confirmed in this study. The increased cholesterol level during alcohol ingestion may be attributed to induction of alpha-hydroxyl methyl glutaryl CoA (HMG CoA) reductase activity, which is the rate limiting step in cholesterol biosynthesis (31), while fatty liver may be as a result of accumulation of triglycerides (32). Our data suggest that moderate alcohol administration to STZ-diabetic rats produced insignificant effect on the levels of serum urea, creatinine, total bilirubin and enzymes such as LDH, GGT, AST, ALP and α-amylase, when compared to untreated diabetic rats. These results indicate that moderate alcohol intake in the diabetic rats may not be a predisposing factor to kidney or liver damage. It is known that increase in the activities of AST, ALT, ALP and GGT could be as a result of severe damage to the hepatocytes during chemical assaults or metabolic disorders which may cause these enzymes to leak into the plasma (33).

In conclusion, the data from the present study demonstrate that moderate consumption of alcohol did not alter the glycaemic indices such as fasting blood glucose, HbA_{1c} or α-amylase levels of diabetic rats. However, moderate alcohol intake produced an adverse effect on the weight of liver and lipid profiles of diabetic rats. Therefore, this study suggests that moderate alcohol intake by subjects with Type 2 diabetes mellitus should be properly reviewed.

ACKNOWLEDGEMENTS

This study was supported by a three-month visiting fellowship (Ref 3240207975) from TWAS-UNESCO Associateship programme given to one of the authors to visit the Institute of Biochemistry and Biophysics, University of Tehran, Iran.

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