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RESEARCH PAPER

Effect of Oral Administration of Syzygium Cumini Seed Powder on the Hepatic Function- related Enzymes of the Alcoholic Transport Laborers

Shahdat Hossain[1*], Bazlul Karim Chowdhury[2], M Hossain[3], Azizur Rahman[1], Ishtiaq Mahmud[4]

[1]*Department of Biochemistry & Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.*
[2]*Department of Biochemistry, Manikgonj Medical College, Manikgonj, Bangladesh.*
[3]*Department of Dentistry, Shaheed Shuhrawardy Medical College Dental Unit, Dhaka, Bangladesh.*
[4]*Department of Biochemistry & Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh*

Abstract

Background: *Syzygium cumini* (locally known as Kalo Jam) seed powder is being used as folk medicine in various diseases since unknown times in Bangladesh. However, data on the systematic studies in human subjects are rare.

Objective: To determine the effect of *Syzygium cumini* seed powder on the hepatic function-related enzymes of the alcoholic transport laborers.

Methods: Here, we investigated the effects of oral administration of *Syzygium cumini* seed powder on hepatic-function markers that included activities of Gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and renal-function markers such as creatinine and urea along with lipid profile, blood pressure and body mass index (BMI) upon chronic alcoholic human volunteers.

Results: The levels of serum ALT, AST and GGT levels decreased, respectively, by 15.0, 8.5 and 23.0% after oral administration of the *S. cumini* powder in the alcoholic subjects. However, the levels of creatinine and urea were not altered significantly. In contrast, the effects of administration of *S. cumini* on the hepatic ALT, AST and GGT enzymes in the non-alcoholic control subjects were less prominent than those found in the alcoholic subjects. Blood pressure decreased only by 5-4 mmHg, while BMI was not altered in either the alcoholic or the non-alcoholic groups. Furthermore, there were significant reduction in the levels of TC (by 10%) and LDL-C (by >20%) and elevation of HDL-C (by 11%) in the *S. cumini*-prescribed alcoholic subjects. TC and LDL-C levels also decreased in the non-alcoholic subjects, but the rate was not statistically significant.

Conclusions: Our results suggest that *S. cumini* seed extract ameliorated GGT, ALT and the atherosclerotic lipid parameters TC and LDL-C without having a significant side effect on the kidney functions, as indicated by the unaltered levels of serum creatinine levels in the alcoholic human subjects without any effect on BP and BMI of the same subjects. Thus, alcohol drinkers can be advised to intake *S. cumini* seed powder to protect against the oxidative hepatic damage.

Keywords*: Syzygium cumini,* Alcoholism, Hepatic enzymes, Herbal Medicine, Liver disease, Hyperlipidemia.

Introduction

Syzygium cumini is a well-known seasonal fruit of Bangladesh. However, its importance in medicinal/ clinical perspectives is largely unknown. We have previously reported that *S. cumini* seed powder possesses anti-diabetic properties and protects the liver against lipid peroxidation with a concurrent amelioration of hepatocellular status of alcoholic rats.[2] Alcohol is one of the most important causes of liver diseases. In Bangladesh, alcoholism is not a usual practice among the general population as there are social and religious barriers against it. However, the prevalence of alcoholism is increasing day by day particularly in the transport laborers. Thus, this is very

***Correspondence:** Shahdat Hossain, Department of Biochemistry & Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.*
Email: shahdat@juniv.edu
ORCID ID: 0000-0003-2970-2324

usual that laborers who abuse alcohol can develop a condition called alcoholic liver disease and a host of other psycho-behavioral problems associated with alcoholism. Furthermore, we have reported that the oral administration of *S. cumini* seed extract ameliorated the levels of lipid peroxides (LPO) in the brain cerebral cortex of alcoholic rats.[3]

Notably, *S. cumini* seed powder is vastly used as a traditional medicine and is sold even by the street vendors of Bangladesh. However, systematic investigation on the effect of *S. cumini* seed in human alcoholism is rare in Bangladesh. After having data at the basic research levels, in vitro and in vivo alcoholic animal studies, our objectives were to examine whether the oral administration of *S. cumini* seed powder ameliorates the hepatic enzymes of the alcoholic human subjects. Though Bangladesh, being a Muslim majority country, is a conservative nation and alcohol is not easy to get, the alcohol consumption still is increasing here. The incidence of alcoholism-related diseases is also increasing particularly in the impoverished area. Our target people in this study were transport-related bus conductors/laborers. This selection was due to the fact that they are increasingly being accustomed to drink alcohols either in crude beverage form such as Tani, Cholai, Dhochuani, Pochani, Chobichi or in imported pure form and are being subjected to a variety of liver pathologies. On the other hand, road traffic accidents among commercial vehicles have been a frequent occurrence in our country. There is need for public awareness campaigns on road safety education and devastating health consequences of alcoholism among commercial motor drivers. Thus, one of the specific objectives of the present investigation was to test the protective effect of *S. cumini* on liver function-related enzymes such as GGT, ALT and AST of the alcoholics. We, therefore, carried out this study with an intention whether oral administration of the *S. cumini* seed powder to the alcoholic transport workers ameliorate their hepatic-functions related enzymes particularly GGT, ALT and AST.

Materials and Methods

Syzygium cumini (L) seed powder and powder-filled capsule was locally collected from local Herbal Company (Bangladesh Herbal and Nutrition Research Limited, Savar, Dhaka). Each capsule contained 500 mg of sterile fine powder.

A total 33 male alcoholic transport workers were used as alcoholic subjects (from the area of Gabtoli and Savar Bus Stand) and 10 non-alcoholic subjects were selected as controls in the study. Average age of the alcoholics was 47±1.5y, while that of the non-alcoholic subjects was 48±2.0 y. They were explained about the study and after getting their written consent, they were selected. Detailed information (age, sex, occupation, educational status, marital status, family history and drug history) was taken from the subjects. If any medication was being taken by the subjects, they were excluded. Other exclusion criteria were acute illness, known serious chronic disease and chronic renal failure diseases and hypertension. *Syzygium cumini* seed powder (500 mg/day/thrice) in the form of capsule was prescribed for 30 days to both the alcoholic and non-alcoholic subjects. All parameters were measured before and after ingestion of capsules. Prior to human studies, *in vitro, ex vivo* and *in vivo* animal studies were conducted and the total polyphenol contents, DPPH-free radical scavenging activities, anti-lipid peroxidation effects in the liver and brain tissues, effects on diabetes and locomotor activities of rats fed *S. cumini* powder were determined and reported.[1-3]

The subjects were allowed to sit for at least 5 minutes to relax while not moving or speaking. The arm was supported at the level of the heart to ensure no tight clothing constricts the arm. The cuff was placed on neatly with the center of the bladder over the brachial artery. The bladder was encircled at least 80% of the arm (but not more than 100%) Both systolic and diastolic blood pressure (BP) was measured using sphygmomanometer by trained personnel. Body weight and height in meter was taken to calculate the BMI.

The levels of G-glutamyl transferase (GGT), alanine aminotransferase (ALT/GPT) and aspartate aminotransferase (AST/GOT) in serum were determined by the commercially available kits (Human Gasellschaft fiir Biochemica Diagnostica, Germany). Plasma urea was estimated by enzymatic, colorimetric, endpoint — Berthelot method. Plasma creatinine was estimated by alkaline picrate method and plasma uric acid was estimated by uricase colorimetric method. Analyses were done by semi-auto biochemical analyzer using commercially available kits. All the tests were carried out as early as possible. Plasma total cholesterol (TC) was

measured enzymatically using the cholesterol oxidase assay with a commercially available kit (Randox Laboratories, Antrium, UK). High density lipoprotein-cholesterol (HDL-C) was measured using the same technique with an HDL-C assay kit (Randox Laboratories) after LDL and very low density lipoprotein (VLDL) had been precipitated with magnesium sulphate and phosphotungstic acid. Plasma triglyceride (TG) was also measured using commercially available kits (Randox Laboratories).

Results are expressed as mean ± SEM (standard error of means). Statistical analysis was done using StatView program (MindVision Software; Abacus Concepts, Berkeley, CA, USA). Comparison of variables between two groups was performed with student's t-test for continuous variables. P values <0.05 were considered statistically.

Results

Prior to *S. cumini*-prescription, there was no statistically significant difference in the baseline BMI of the non-alcoholic versus alcoholic subjects [BMI of non-alcoholic vs. alcoholics: 23.97±0.57, 24.56±1.42]. Prior to *S. cumini*-prescription, the basal systolic blood pressure (SBP), however, was significantly higher in the alcoholic subjects [non-alcoholic control vs. alcoholic subjects [SBP: 110±3.56, 119.29±4.68 mm Hg]. However, the diastolic blood pressure was not significantly different in the alcoholic subjects when compared to that of the nonalcoholic control subjects [Non-alcoholics vs. Alcoholics, DBP: 73.75±2.63, 70.62±2.73 mmHg].

Effects of prescription of *S. cumini* powder on the BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP) of alcoholic subjects are shown in the Figure 1. Oral administration of *S. cumini* powder did not affect the BMI of either alcoholic or non-alcoholic subjects. The systolic blood pressure (SBP) decreased by ~ 5-6 mmHg, while the diastolic blood pressure (DBP) decreased by 2-3 mmHg in the *S. cumini*-prescribed alcoholic subjects, however, the hypotensive effects were not statistically significant. The hypotensive effect *S. cumini* powder was less prominent in the control non-alcoholic subjects.

Before the treatment began, the basal levels of the GGT, ALT and AST also were significantly higher in the alcoholic subjects than those of the non-alcoholic subjects. The effects of prescription of *S. cumini* powder on the hepatic-function marker GGT, ALT and AST are shown in the Figure 2. The oral administration of *S. cumini* powder for 30 days to the alcoholic subjects significantly decreased the serum levels of GGT (by 23.3%), ALT (by 15.2%). The effect on the AST levels was not statistically significant, though it decreased by 8.5%.

The effects of prescription of *S. cumini* powder on the levels of urea, creatinine and uric acid are shown in the Figure 3. After oral administration of *S. cumini*, the levels of blood urea nitrogen, blood creatinine, and uric acid increased in the non-alcoholic control subjects, however, the values were not statistically significant. The levels of these parameters also were not altered in the *S. cumini*-prescribed alcoholic

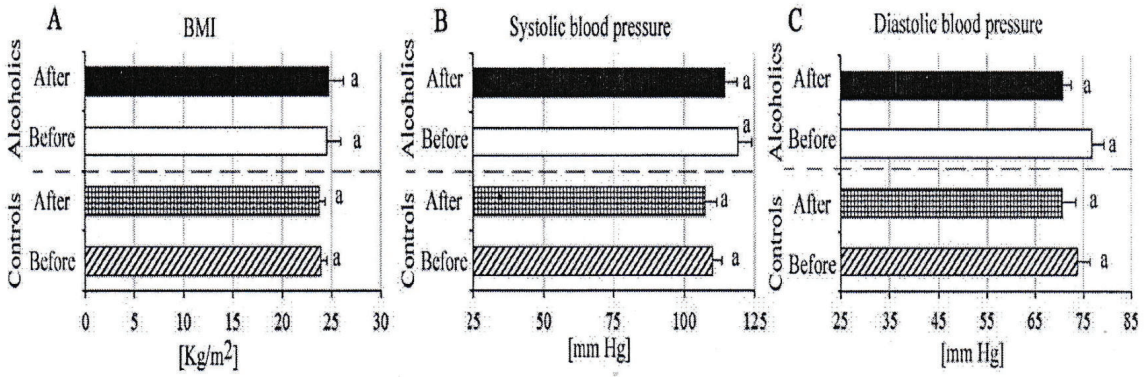

Figure 1: Effect of oral administration of *S. cumini* seed powder on the BMI (A), SBP (B) and DBP (C) of the control (non-alcoholic, n = 10) and alcoholic (n=33) subjects. Before: Measured before administration of S. *cumini* seed powder. After: Measured after administration of S. *cumini* seed powder. Results are mean +SEM (standard error of means) (n=33). Bars that share a common alphabets are not significantly different at P<0.05 (One-Way ANOVA).

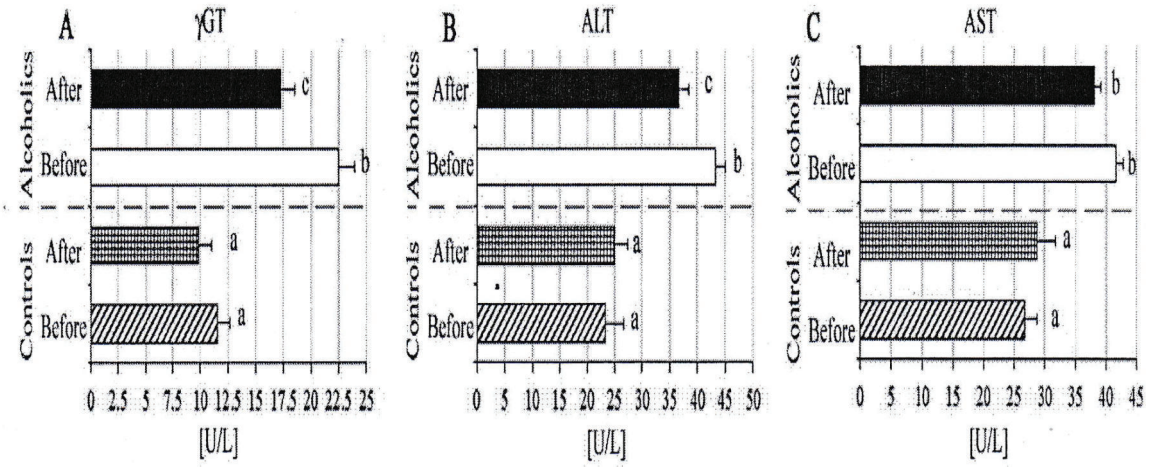

Figure 2: Effect of oral administration of *S. cumini* seed powder on the serum Q' -GT, ALT and AST levels of the control (nein-alcoholic, n'— 10) and alcoholic (n=33) subjects. Enzyme activities were determined before and after oral administration of *S. cumini* seed powder. Results are mean ± SEM (standard error of means). Bars with different alphabets are significantly different at P<0.05 (One-Way ANOVA).

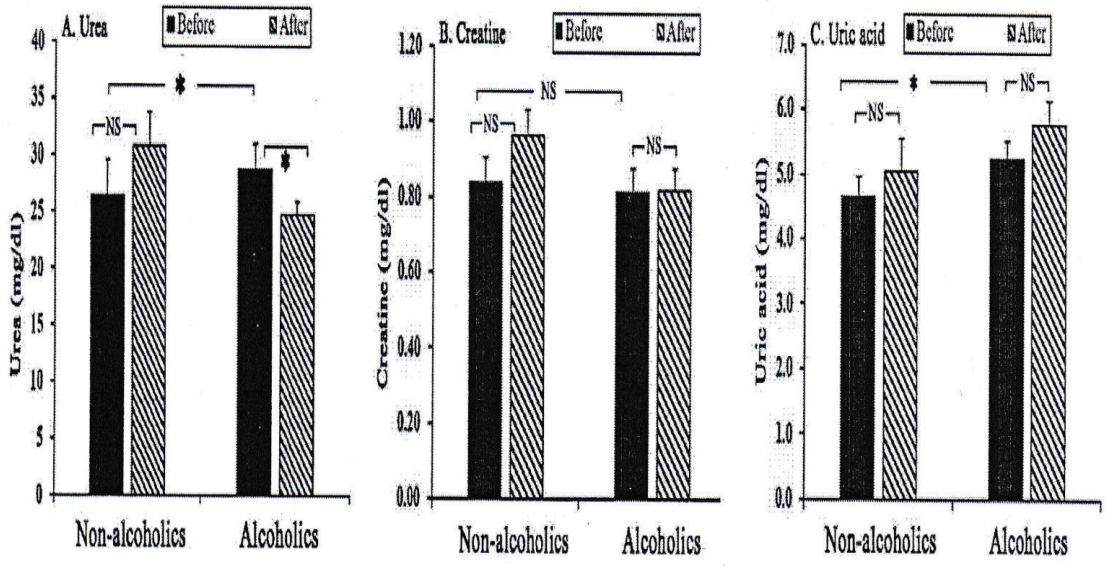

Figure 3: Effect of oral administration of *S. cumini* seed powder on the serum urea, creatinine and uric acid levels of the control (non-alcoholic, n = 10) and alcoholic (n=33) subjects. Results are mean ± SEM (standard error of means). Bars with symbols [*] are significantly different at p<0.05 (One-Way ANOVA). NS: Not significant.

subjects before and after treatment, except the urea levels, which significantly decreased after *S. cumini* treatment.

The effects of oral administration of the S. cumini powder on the lipid profile are shown in the Table I.

The oral administration of the S. cumini powder significantly decreased the levels of total cholesterol (TC) in the serum of alcoholic subjects but not in the non-alcoholic subjects. The levels of TG were not altered either in the alcoholic or non-alcoholic subjects. The levels of HDL-C significantly increased (by 11%), while those of the LDL-C significantly decreased (by 20.4%) in the alcoholics but not in the non-alcoholics. These resulted in a significant increase in the ratios of HDL-C/LDL-C in both the alcoholic and nonalcoholic subjects.

Table I: Effect of *S. cumini* seed powder on plasma lipid profile of alcoholic and non-alcoholic subjects

Parameters	Alcoholic subjects			Non-alcoholic subjects(Controls)		
	Before	After	%△	Before	After	%△
Total cholesterol	164 ± 6.50[a]	147 ±7.10	-10.4↓	141 ± 4.8	138±	-2.1↓
Triacylglycerol	146 ± 8.10a	139 ±8.20	-4.8.0	114±	123±	+7.8↑
HDL-C (mg/dl)	31.5 ± 1.20	35 ± 1.70 b	+11.0	31±	28.0±	-9.6↓
LDL-C (mg/dl)	103 ± 6.0	82.0±	-20.4↓	87.0±	85.0 ± 9.1	-2.3↓
HDL-C/LDL-C	0.322 ± 0.02	0.502±0.06	+55%↑	0.372±0.04[a]	0.311±0.03[a]	-16.4↓

Results are mean ± SEM (standard error of means), n=33 for alcoholics and n=10 for the non-alcoholics. Parameters were determined before and after oral administration of *S. cumini.* Values in the same row (either in the alcoholic or non-alcoholic groups) that do not share a common superscripts are significantly different at $p<0.05$ (student's t-test). Here, %△ = Percent change between *before* and *after* the treatment of *S. cumini.*

+= Plus sign indicates increase. - = Minus indicates decrease.

Discussion

The results of the present study clearly suggest that the levels of hepatic marker enzymes, including, GGT, ALT and AST were higher in the alcoholic transport workers than those of the normal control subjects. Alcoholic liver disease remains a major cause of morbidity and mortality worldwide. The results of our investigation are qualitatively consistent with other reports where liver functions were deteriorated in the chronic alcohol drinkers.[4, 5,6]

The exact mechanism through which the serum GGT increased and as well as it reduced after oral administration of *S. cumini* powder in the serum of the alcoholic-subjects is not clearly understood. Gamma-glutamyl transferase (GGT) catalyzes the transfer of gamma-glutamyl functional groups from glutathione (GSH) to an acceptor that may be an amino acid, a peptide or water (forming glutamate).[7] GGT plays a key role in the degradation of glutathione[8], thus conferring it to exert a pro-oxidant role.[9] GGT is remarkably present in the liver tissues, which is vigorously involved in the xenobiotics Metabolism, influx-efflux of biles/biliary components and lipid absorption, and thus the status of the GGT reflects the functional status of the livers and enables to act as a diagnostic marker for liver disease. High levels of GGT indicate alcohol toxicity.' Therefore, an increased level of GGT in the serum of the alcoholic subjects is consistent with these reports. The alcohol consumption also histologically deteriorated the morphologies of liver cells in rat model. All these evidence are thus consistent with increased levels of GGT in our present investigation. We have previously reported that chronic alcohol-administration to rats increased the levels of hepatic lipid peroxides (LPO) with a concurrent decrease in the levels of GSH in the liver tissues.[2] The results are consistent with the increased ability of alcohol to produce free radical-induced lipid peroxidation and decline the hepatic *levels of GSH.*[11] *Therefore,* we *hypothesize* that alcohol might have oxidatively disrupted or deteriorated the hepatic cell, and hence the GGT levels increased in the serum of the alcoholic subjects of our present investigation.

Usually, the ALT and AST enzymes are required to metabolize proteins in the liver cells. If the liver is inflamed/damaged, there will usually be high levels of ALT/AST in the blood stream. Bell et al. (1987) reported that the serum AST and ALT are less often elevated than GGT in the alcoholic subjects.[11] In our investigation, GGT increased by 94%, while AST and ALT increased, respectively by —55 and 85 % in the alcoholic subjects. Thus our results of increased levels of AST and ALT in the alcoholic subjects are consistent with their reports. Since the serum AST or ALT can also arise from non-hepatic sites, particularly heart and muscle and levels are increased in conditions such as myocardial infarction and skeletal muscle trauma, therefore, the increased levels of ALT and AST are claimed to be of limited sensitivity.[12,13] However, it is claimed that the ratio of AST to ALT in serum may also help in the diagnosis of some liver diseases. In most patients with acute liver injury, this ratio is 1 or less, whereas in alcoholic hepatitis, it is generally about 2.[14] Before treatment with *S. cumini* seed powder, the AST:ALT ratio was 0.96±0.05 in the alcoholics, whereas it was 1.25±0.10 in the non-alcoholic subjects in our investigation. The decreased

ratios of the AST/ALT enzymes (which was < 1.0) are thus consistent with the indications of the acute liver disease. Finally, as a whole, the increases in the levels of GGT, ALT and AST in the alcoholic subjects are consistent with the pathological states of their liver, and if untreated, they may suffer from serious hepatic diseases including cirrhosis.

Therefore, the oral administration of *S. cumini* for 30 days and the resultant amelioration of GGT, ALT and AST might be related to the anti-oxidative effects of the *S. cumini* on the hepatocellular integrity of the bilayer plasma membranes.[2,13,14] The treatment with *S. cumini* also improved the cellular morphology, reduced membrane leakage and subsequently decreased the GGT, ALT and AST enzyme levels in the serum of the *S.* cumini-treated alcoholic subjects of the present study. We have previously reported that the *S. cumini* powder extract inhibits *in vitro* oxidative stress and results in reduced levels of lipid peroxide. Inhibition of *S. cumini* to the *in vivo* production of lipid peroxide in the liver of the alcoholic rats was also reported.[2] Therefore, it is speculated that prescription of *S. cumini* powder with its both anti-oxidant and anti-inflammatory properties attenuated the adverse morphological/biochemical changes induced by acute alcohol drinking. The exact mechanism(s) of the ameliorative actions of *S. cumini* seed still remains to be known clearly. *S. cumini* seed extract contains 13 mg gallic acid equivalent phenolic compounds/g dry powder including catechins which scavenge free radicals.[2] It is thus suggestive that the anti-oxidative agents present in *S. cumini* seed reduced the oxidative stress or indirectly might have provoked an anti-oxidative enzyme defense in the liver tissues of the alcoholic subjects. The speculation is consistent with the reports of others, where the administration of *S. cumini* seed extract increased the levels of anti-oxidative enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase as well as reduced glutathione (GSH) in rats.[15] Thus, there may be the possibility of activation of some of the endogenous anti-oxidative enzymes in the present study.

Physicians used to focus attention on potential toxicological effects; though identification of toxicological effects of herbal preparations has been often difficult as patients generally self-medicate with these and withhold information. Kidney failure patients experience a high creatinine level as failed kidneys cannot discharge excess creatinine out of the blood timely. Kidneys are the organs responsible for keeping serum creatinine within the normal range. When they are affected, kidney functions decrease and creatinine level increases. Therefore, we also measured the serum creatinine level both in the alcoholic and in non-alcoholic subjects to assess whether the oral administration of the *S. cumini* powder exerts any detrimental effect on the kidneys. Notably, we have previously reported that *S. cumini* feeding to alcoholic rats ameliorated in the morphology of kidneys and liver of alcoholic rats.[23] In view of its non-toxic nature, *S. cumini* might be developed as an effective therapeutic agent against alcohol-induced liver disease by its anti-oxidative and anti-inflammatory features.

Other markers, including plasma total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and triglyceride (TG) may be correlated with alcohol consumption. Ethanol consumption activates hepatic *de novo* lipogenesis and the major quantitative fate of ethanol is the production of acetate in the liver. The acetate released into the plasma inhibits lipolysis in peripheral tissues by 53% and whole body lipid oxidation by 73%. The pathogenesis of alcoholic fatty liver and alcoholic hyperlipidemia has been known for a long time to be due mainly to a combination of decreased fatty acid oxidation in mitochondria and to increased glycerolipid synthesis.'[6] Hyperlipidemia associated with alcohol consumption is relevant to the problem of atherosclerosis and heart disease in the alcohol-drinking population. In the general population, elevations in LDL-C are correlated with increased risk of coronary artery disease. Increases in HDL-C are associated protection. Elevated serum TGs have been identified as an independent risk factor for cardiovascular diseases. Therefore, we evaluated the effect of *S. cumini* seed powder on the lipid profiles of both the alcoholic and non-alcoholic subjects before and after the treatment. Prior to the administration of the *S. cumini* powder total cholesterol levels were significantly higher in the alcoholic subjects (164±3.4 mg/dl), as compared to those of the non-alcoholic control subjects (141±3.5 mg/dl) (Table I). The LDL-C levels were >18% higher in the alcoholics than those of the non-alcoholic subjects (Table I). Thus, the increased levels of serum TC and/or LDL-C were consistent with the impaired cholesterol metabolism

in the alcoholics. Serum levels of independent risk factor for cardiovascular disease i.e. TG were also higher in the alcoholic subjects (146 vs. 114 mg/dl) (Table I). Oral administration of *S. cumini* powder for 30 days decreased the levels of TC by 10.1% ($p<0.05$), TG by 5.2% (P=not significant) (Table I). Levels of LDL-C, the most atherogenic lipid, significantly decreased (by >20%), while HDL-C levels increased by 11% after oral administration of *S. cumini* to the alcoholic subjects (Table I). *S. cumini* on decreased the lipid profile of the non-alcoholic subjects, however, the values did not reach significance. Oral administration of *S. cumini* seed powder thus displayed both the anti-hepatotoxic as well as anti-hyperlipidemic effects in the alcoholic subjects.

Conclusion

Syzygium cumini seed powder extract reproducibly exhibits beneficial effects on the hepatorenal functions and histologies of alcohol-consumed animal models. On the basis of the basic research results, we prescribed *S. cumini* powder to the alcoholic transport workers suffering from vulnerable liver damages and observed ameliorating effects. This study might have an important impact on the alcoholic individuals of Bangladesh, who are suffering from hepatic dysfunctions. On the other hand, Bangladesh is a poor economic country, where a high quality, reliable and affordable basic medical healthcare service for her people has remained a challenge. Therefore, the chief and safe complementary and alternative medicines might play important roles in the management of the alcoholism-related diseases in the poor country like Bangladesh. Finally, *S. cumini* seed can help in lessening, at least in part, the economic burden involved in the management of alcoholism-associated hepatic and hyperlipidemia-instigated cardiovascular diseases. Further extensive study is indeed, necessary to determine the precise mechanism of actions of *S. cumini* seed powder on impaired hepatic functions. The study, however, provides a clear cut evidence that the alcohol thinkers can be advised to intake *S. cumini* seed powder instead of ingesting high priced anti-oxidants to protect against the oxidative hepatic damage. Therefore, the fact that the patients, including transport workers with chronic liver disease might be prescribed for their liver diseases, which involves alcohol-induced both liver injury and hyperlipidemia.

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