Effect of Pioglitazone, Quercetin, and Hydroxy Citric Acid on the Lipid Profile and Lipoproteins in Experimentally Induced Non-alcoholic Steatohepatitis (NASH)

Surapaneni Krishna Mohan[1,*] and Mallika Jainu[2]

[1]Associate Professor, Department of Biochemistry, Saveetha Medical College & Hospital, Faculty of Medicine, Saveetha University, Saveetha Nagar, Thandalam, Chennai – 602 105, Tamilnadu, India
[2]Assistant Professor, Department of Biomedical Engineering, SSN Engineering College, OMR, Klavakkam, Chennai – 603 110, Tamilnadu, India

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ABSTRACT

Background: Non-alcoholic steatohepatitis (NASH) is an emerging disease belonging to the non-alcoholic fatty liver disease (NAFLD) spectrum, which may progress to fibrosis and thereby to cirrhosis of the liver. Currently, no definitive and effective treatment strategies have been identified to treat NASH. **Objective:** To study the effect of pioglitazone, quercetin, and hydroxy citric acid on lipid profile parameters and lipoproteins in experimentally induced non-alcoholic steatohepatitis (NASH). **Materials & Methods**: NASH was induced in the rats by feeding them the high-fat diet for eight weeks. In drug treated groups, after the rats were fed with high-fat diet for four weeks, they were treated in conjunction with intragastric administration of pioglitazone (4mg/kg body wt), quercetin (20mg/kg body wt) and hydroxy citric acid (150 mg/kg body wt) for an additional four weeks. The concentration of total cholesterol, free cholesterol, esterified cholesterol, phospholipids, triglycerides, free fatty acids (FFAs), high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins (VLDL), were studied. **Results:** The experimentally induced-NASH rats treated with pioglitazone, quercetin, and hydroxy citric acid showed marked reduction in the concentration of lipid profile and lipoproteins when compared with that of the NASH-induced group, where as quercetin reversed the changes in a significant manner compared with pioglitazone & HCA. **Conclusion**: The protective effect of pioglitazone, quercetin, and hydroxy citric acid was observed via the decrease of lipoprotein and lipid concentrations towards normal ranges in the drug-treated groups.

Keywords: Non-alcoholic steatohepatitis, lipid profile, lipoproteins, quercetin, hydroxy citric acid, pioglitazone.

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***Address for correspondence:**
Dr. Surapaneni Krishna Mohan,
Associate Professor, Department of Biochemistry, Saveetha Medical College & Hospital, Faculty of Medicine, Saveetha University, Saveetha Nagar, Thandalam, Chennai – 602 105, Tamilnadu, India.
E-mail: krishnamohan.surapaneni@gmail.com

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is an asymptomatic disease that may lead to end-stage liver disease if not diagnosed and treated.[1–6] NASH affects both adults and children, and the prevalence of NASH is increasing yearly, leading to significant morbidity.[7,8] NASH is often associated with metabolic syndrome, even though multiple pathogenic mechanisms have been suggested.[9–11] Although the aetiology is unknown, NASH is most frequently observed in people with one or more of the following conditions: type

2 diabetes mellitus, insulin resistance, obesity, and hypertriglyceridaemia.[12]

Recently, human and animal studies have addressed key issues in NASH pathogenesis, such as the nature and cause of insulin resistance, the role of insulin resistance in inflammation, liver cell injury, and free fatty acid accumulation, the mechanisms for inflammatory recruitment and perpetuation, the biochemical basis and significance of oxidative stress, and the pathogenesis of fibrosis.[13,14]

Since the pathogenesis of NASH involved interplay of 3 possible mechanisms[15] such as hyper-insulinemia, lipotoxicity and oxidative stress, we have chosen 3 categories of drugs, pioglitazone as insulin sensitizer, quercetin as hepatoprotectant & antioxidant and hydroxy citric acid (HCA) as a lipid lowering agent & anti-obesity agent.[16]

In the present study, the concentration of serum lipid profile parameters, such as total cholesterol, free cholesterol, esterified cholesterol, phospholipids, triglycerides, and FFAs, were elevated significantly in experimentally induced-NASH rats (group 2) when compared with the control group (group 1).

MATERIALS & METHODS

NASH was induced in rats by feeding a high-fat diet for eight weeks,[17] and this model was used to conduct a comparative study of the role of pioglitazone, quercetin, and hydroxy citric acid on various parameters in NASH. Male Wistar rats weighing approximately 150g were housed in solid-bottomed polypropylene cages under strict veterinary supervision and maintained in rooms with a controlled 12h light/dark cycle. The animals received a standard diet or high-fat diet and water *ad libitum* as per the experimental protocol.

Pioglitazone hydrochloride is a widely used drug in the treatment of insulin resistance diabetes.[16] Quercetin is a phytochemical and a known flavonoid.[16] (-)-HCA was well known chemical compound for its regulatory effect on fatty acid synthesis, lipogenesis, appetite, and weight loss.[16]

This study conformed to the guiding principles of the Institutional Animal Ethical Committee (IAEC), Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), and the Guide for the Care and Use of Laboratory Animals (IAEC Approval Number: 001/006/2010 & 01/007/2011).

The Male Wistar rats selected for the study were divided into eight groups:[18,19]

Group 1; Controls (n = 6): Control rats received the regular standard diet for eight weeks.

Group 2; NASH (n = 6): NASH was induced in the rats by feeding them the high-fat diet for eight weeks.

Group 3; Pioglitazone Control (n = 6): After the rats were fed the standard diet for four weeks, they were fed the standard diet in conjunction with intragastric administration of pioglitazone (4mg/kg body wt) (0.5% methyl cellulose w/v) for an additional four weeks.

Group 4; Quercetin Control (n = 6): After the rats were fed the standard diet for four weeks, they were fed the standard diet in conjunction with intragastric administration of quercetin (20mg/kg body wt) dissolved in 1% DMSO (v/v) for an additional four weeks.

Group 5; Hydroxy Citric Acid Control (n = 6): After the rats were fed the standard diet for four weeks, they were fed the standard diet in conjunction with intragastric administration of hydroxy citric acid (150mg/kg body wt) for an additional four weeks.

Group 6; NASH+Pioglitazone (n = 6): After the rats were fed the high-fat diet for four weeks, they were fed the high-fat diet in conjunction with intragastric administration of pioglitazone (4mg/kg body wt) (0.5% methyl cellulose w/v) for an additional four weeks.

Group 7; NASH+Quercetin (n = 6): After the rats were fed the high-fat diet for four weeks, they were fed the high-fat diet in conjunction with intragastric administration of quercetin (20mg/kg body wt) dissolved in 1% DMSO (v/v) for an additional four weeks.

Group 8; NASH+Hydroxy Citric Acid (n = 6): After the rats were fed the high-fat diet for four weeks, they were feed a high-fat diet in conjunction with intragastric administration of hydroxy citric acid (150mg/kg body wt) for an additional four weeks.

After the experimental period, the animals were fasted for 12h and then sacrificed by cervical decapitation. The blood was collected from jugular vein, centrifuged for 5min at 3000rpm/min, and the serum was stored at −70°C until the various biochemical analyses were conducted. The total cholesterol concentration was determined by the method of Parekh and Jung.[20] The free cholesterol content was fractionated using digitonin according to the method of Leffler and McDouglad.[21] The esterified cholesterol concentration was derived by calculating the difference between the total and free cholesterol. The phospholipid content was determined by the method of Zilversmith and Davis.[22] The triglyceride concentration was assessed by the method of Rice.[23] The free fatty acid content was determined using the method described by Hron and Menahen and the colourimetric assay based on the method of Itaya.[24] The lipoproteins were fractionated by a dual precipitation technique, as described by Wilson and Spiger.[25] The total high density lipoproteins

(HDL) were separated according to the method of Burnstein *et al.*[26] All these parameters were estimated by using autoanalyser.

RESULTS

Fig. 1–6 show the effect of pioglitazone, quercetin, and hydroxy citric acid on the lipid profile of experimentally induced-NASH rats. The concentrations of serum lipid profile parameters, such as total cholesterol, free cholesterol, esterified cholesterol, phospholipids, triglycerides, and FFAs were elevated significantly in the experimentally induced-NASH rats (group 2) compared with the control group (group 1). The experimental NASH rats treated with pioglitazone (group 6; NASH + pioglitazone), quercetin (group 7; NASH + quercetin), and hydroxy citric acid (group 8; NASH + HCA) showed a marked reduction in the concentration of lipid profile parameters compared with the un-treated NASH rats (group 2). Compared with the control group (group 1), the concentration of total cholesterol, esterified cholesterol, phospholipids, and FFAs did not exhibit a

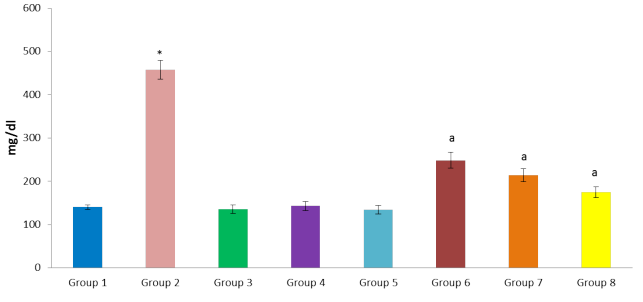

Figure 1: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentration of total cholesterol in experimental NASH.
*P<0.001 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

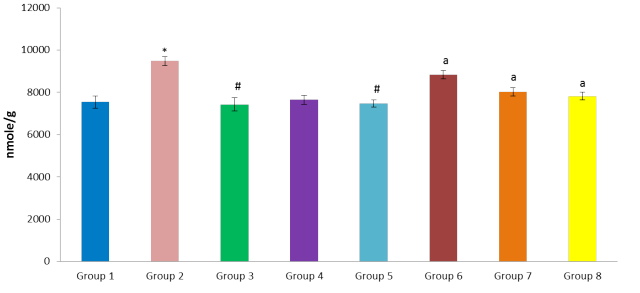

Figure 2: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentration of free cholesterol in experimental NASH.
*P<0.001 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

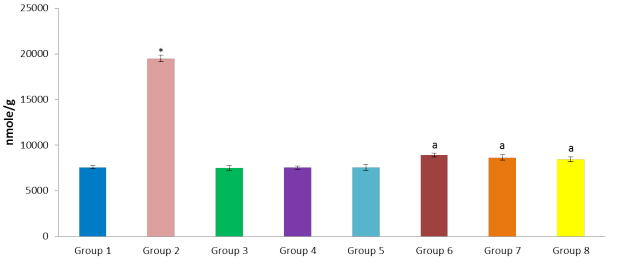

Figure 3: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentration of esterified cholesterol in experimental NASH.
*P<0.001 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

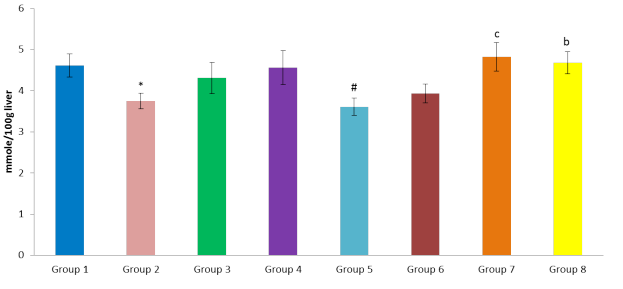

Figure 4: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentration of phospholipids in experimental NASH.
*P<0.001 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

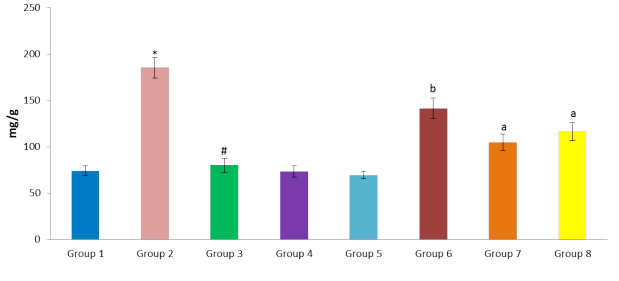

Figure 5: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentration of triglycerides in experimental NASH.
*P<0.001 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

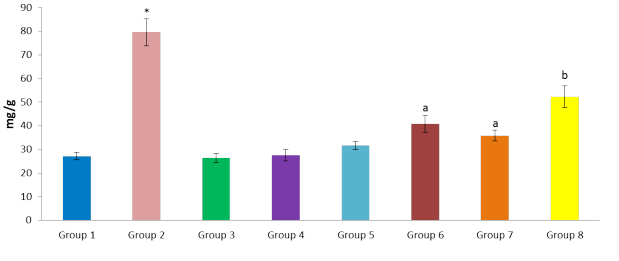

Figure 6: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentration of free fatty acids (FFAs) in experimental NASH.
*P<0.001 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

significant change in the rats fed the standard diet in conjunction with pioglitazone (group 3; pioglitazone control); however, the pioglitazone-treated rats did demonstrate a significant reduction in free cholesterol and triglyceride concentrations. Additionally, a significant effect was not observed in the lipid profile parameters of the rats fed the standard diet in conjunction with quercetin (group 4; quercetin control), compared with the control group (group 1); whereas, compared with the control group (group 1), the concentration of free cholesterol and phospholipids were significantly reduced in the rats fed the standard diet in conjunction with hydroxy citric acid (group 5; HCA control), while the total cholesterol, esterified cholesterol, triglycerides, and free fatty acid concentrations did not demonstrate a significant change. The concentration of lipoproteins, such as HDL, LDL, VLDL, HDL:LDL, and TC:HDL, are presented in Fig. 7–11. Increased concentrations of low-density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol are biomarkers of

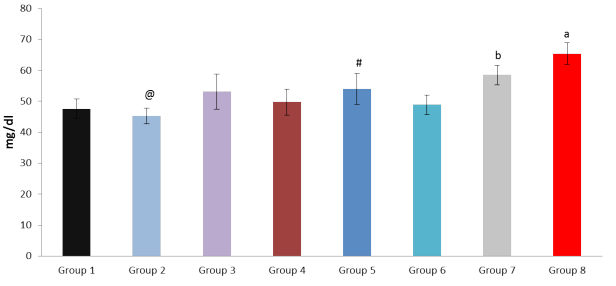

Figure 7: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentration of high density lipoproteins (HDL) in experimental NASH.
*P<0.001 and @P<0.05 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

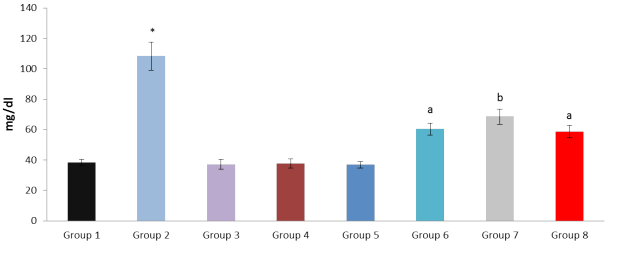

Figure 8: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentration of low-density lipoproteins (LDL) in experimental NASH.
*P<0.001 and @P<0.05 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

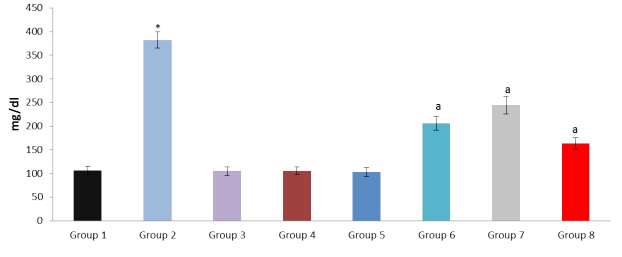

Figure 9: Effect of pioglitazone, quercetin, and hydroxy citric acid on the concentrations of very low-density lipoproteins (VLDL) in experimental NASH.
*P<0.001 and @P<0.05 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

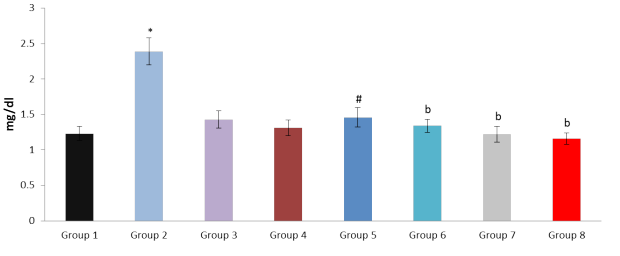

Figure 10: Effect of pioglitazone, quercetin, and hydroxy citric acid on the ratio of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) in experimental NASH.
*P<0.001 and @P<0.05 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

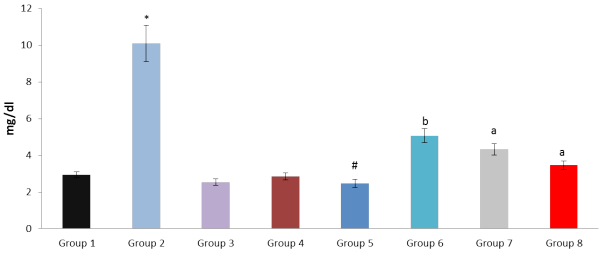

Figure 11: Effect of pioglitazone, quercetin, and hydroxy citric acid on the ratio of total cholesterol (TC) and high-density lipoproteins (HDL) in experimental NASH.
*P<0.001 and @P<0.05 compared with control group; [a]P<0.001 compared with NASH group; [b]P<0.01 compared with NASH group; [c]P<0.05 compared with NASH group; [#]P<0.001 compared with control group.

insulin resistance. In this study, the disease progression was directly proportional to the lipoprotein levels, which was supported by a significant increase in lipoprotein concentrations in the experimentally induced-NASH group (group 2) compared with the control rats (group 1). Compared with the experimentally induced-NASH group (group 2), the lipoprotein levels decreased towards the normal range in group 6 (NASH + pioglitazone), group 7 (NASH + quercetin), and group 8 (NASH + HCA), which demonstrated the protective effect of pioglitazone, quercetin, and hydroxy citric acid.

DISCUSSION

In the present study, increased levels of LDL cholesterol and low levels of HDL cholesterol are biomarkers of insulin resistance. The disease progression was directly proportional to the lipoprotein levels, which was supported by a significant increase in lipoprotein levels in the experimentally induced-NASH group (group 2) compared with the control rats (group 1).

NASH has been observed to be associated with atherogenic lipid profiles, including hypertriglyceridaemia, a higher plasma concentration of large VLDL and LDL, and lower HDL concentrations.[27,28] The presence of increased circulating and/or hepatic saturated fatty acids might promote the development and progression of liver damage, which subsequently activates apoptosis.[29,30,31] Moreover, in the liver, the increase of fatty acid synthesis with the concomitant degradation of apolipoprotein B100, which reduces fatty acid delivery from hepatocytes via VLDL, causes an imbalance of hepatic fat turnover, resulting in steatosis.[32,33,34]

The present study revealed a significant dyslipidaemia (lower HDL-c, higher total cholesterol, LDL-c, and triglycerides) in the experimental NASH rats. Our observations were supported by other studies documented in the literature.[35,36]

To analyse the individual effects of pioglitazone, quercetin, and hydroxy citric acid on the liver and on normal metabolic activities, we studied three drug control groups. Pioglitazone treatment (group 3; pioglitazone control) in conjunction with the standard diet did not produce a significant effect on total cholesterol, esterified cholesterol, phospholipids, and FFA concentrations but resulted in a significant reduction in free cholesterol and triglyceride levels, compared with the control rats (group 1). This result could be attributed to the hypoglycaemic drug pioglitazone, which modulates the levels of lipids.[37] However, compared with the control rats (group 1), quercetin treatment (group 4; quercetin control) in conjunction with the standard diet did not produce a significant effect on any of the lipid profile parameters. Hydroxy citric acid treatment (group 5; HCA control) in combination with the standard diet did not produce a significant effect on total cholesterol, esterified cholesterol, triglycerides, and FFA concentrations but resulted in a significant reduction in free cholesterol and phospholipids concentrations, compared with the control rats (group 1). This result could be attributed to the lipid lowering action of HCA.[38,39]

Additionally, pioglitazone (group 3; pioglitazone control) and quercetin (group 4; quercetin control) treatment in combination with the standard diet did not produce a significant effect on lipoprotein levels, compared with the control rats (group 1). However, although the hydroxy citric acid treatment (group 5; HCA control) in conjunction with the standard diet did not significantly affect LDL and VLDL levels, the group 5 rats did demonstrate a significant increase in HDL levels compared with the control rats (group 1). This result could be attributed to the lipid lowering and hypocholesterolaemic action of HCA.[38,39]

We determined that pioglitazone was effective in reducing serum total cholesterol and LDL values. Patients with insulin resistance have impaired insulin responses in muscle, adipose tissue, and the liver, causing compensatory increases in pancreatic insulin secretion to maintain glucose levels within the normal range. Chronic hyperinsulinaemia causes triglycerides to accumulate in hepatocytes by favouring the formation of triglycerides instead of mitochondrial beta-oxidation yet impairing the secretion of triglycerides into the circulation. Compounding this dysfunctional metabolic handling of fat in the liver is the continued release of FFAs by peripheral adipose tissue in the fed state because of insulin resistance at the level of the adipocytes.[40,41]

Lipid peroxidation and oxidant stress have been proposed as an important link between the accumulation of fat and subsequent liver injury.[42] Quercetin could improve the outcome of NASH because it reduces lipid levels, limits oxidative stress, and modulates inflammatory responses. In this study, the improved lipid profile, increased insulin sensitivity, and lower fasting plasma glucose values suggest that quercetin supplementation induces the regression of NASH. In addition, this flavonoid can also reduce the number of adipocytes by decreasing adipogenesis or increasing apoptosis. Kobori et al. (2011) reported that the chronic dietary intake of quercetin resulted in reduced body weight gain as well as reduced visceral and liver fat accumulation and improved systemic parameters related to metabolic syndrome (hyperglycemia, hyperinsulinemia, and dyslipidemia), most likely by decreasing oxidative stress.[43]

In the present study, quercetin exhibited significant hypolipidaemic and hypocholesterolaemic activities.

Following the treatment with quercetin, the elevated LDL-C levels significantly reduced among the rats with NASH. These data might reflect the antioxidant property of quercetin and the increased concentration of HDL-C, which is capable of inhibiting LDL-C peroxidation and retarding LDL-C accumulation. Remarkably, serum HDL-C levels increased more than 50% in quercetin-treated experimental rats.[44] As a multifunctional lipoprotein, HDL-C possess antioxidant and anti-inflammatory activities.[45]

(-)-HCA is a potent inhibitor of ATP citrate lyase, which catalyses the extra mitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA. The inhibition of this reaction limits the availability of acetyl-CoA units required for fatty acid synthesis and lipogenesis during a lipogenic diet, that is, a diet high in carbohydrates.[46] Extensive animal studies have indicated that (-)-HCA suppresses fatty acid synthesis, lipogenesis, and food intake and induces weight loss.[47] Experiments using (-)-HCA have demonstrated that the citrate cleavage enzyme is an obligatory enzyme in pyruvate-derived lipogenesis and that the lipogenic system of rabbit adipose tissue resembles a ruminant because it is adapted to utilise acetate rather than glucose.[48] Research has shown that the conversion of carbohydrates into fat is prevented by (-)- HCA[49]. A more important function of HCA is its ability to increase the carnitine palmitoyl transferase-1 (CPT-1) activity by decreasing the pool of acetyl-CoA, thus reducing the level of malonyl-CoA and raising the activity of CPT-1.[50] HCA is also suggested to have an effect on plasma cholesterol via increased plasma HDL-C, and so may also protect LDL from oxidation.[51] In the present study, HCA supplementation significantly reduced plasma triglyceride levels and increased plasma HDL-C.

CONCLUSION

Our study data confirm that quercitin possesses more significant lipid lowering action in NASH condition when the comparison done with HCA and pioglitazone. Moreover, it has hypolipidemic and antioxidant activities, therefore it has an ability to prevent steatosis complications. Hence, above findings have given scientific evidence to the use of quercitin in the treatment of NASH.

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