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NCE-9 Inhibits Protein Tyrosine Phosphatase Ptp1B and Prevents Hypertriglyceridemia and Hepatic Steatosis In Fructose Fed Rats

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ABSTRACT

Fructose consumption from sucrose and high-fructose corn syrup have Increased both plasma insulin and leptin act in the central nervous system in the long-term regulation of energy homeostasis. Because fructose does not stimulate insulin secretion from pancreatic β cells, the consumption of foods and beverages containing fructose produces smaller postprandial insulin excursions than does consumption of glucose-containing carbohydrate. Because leptin production is regulated by insulin responses to meals, fructose consumption also reduces circulating leptin concentrations. The combined effects of lowered circulating leptin and insulin in individuals who consume diets that are high in dietary fructose could therefore increase the likelihood of weight gain and its associated metabolic sequelae. In addition, fructose, compared with glucose, is preferentially metabolized to lipid in the liver. Fructose consumption induces insulin resistance, impaired glucose tolerance, hyper insulinemia, hyper triacylglycerolemia, and hypertension in animal models. The data in humans are less clear. Although there are existing data on the metabolic and endocrine effects of dietary fructose that suggest that increased consumption of fructose may be detrimental in terms of body weight and adiposity and the metabolic indexes associated with the insulin resistance syndrome, much more research is needed to fully understand the metabolic effect of dietary fructose in humans. We investigated the effects of NCE-9 on fructose-induced hypertriglyceridemia and liver steatosis in rats. NCE-9 reduced serum TG and VLDL levels and also improve glucose intolerance in fructose-fed rats. This PTP1B inhibitory property may be a promising therapeutic strategy for NCE-9 to treat fructose-induced hepatic steatosis.

Keywords: NSE-9**,** Protein tyrosine phosphatase 1B **,** Insulin resistant**,** High Fructose Fed, rat**,** Hypertriglyceridemia**,** Hepatic Steatosis

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INTRODUCTION

The sudden surge in the incidence of type 2 diabetes is complicated by the fact that it is multi-factorial disease, frequently associated with a cluster of pathologies including obesity, hypertriglyceridemia, impaired glucose tolerance, insulin resistance and hypertension, collectively referred to as the metabolic syndrome.[1] There has been heightened awareness of the metabolic syndrome and its prevention, due to its strong association with premature morbidity and mortality. In particular, these predisposing factors subject the individual to greater cardiovascular risk and type 2 diabetes.[2] The main driving forces that increase the prevalence of insulin resistance is the dramatic rise in obesity driven by modern Westernized Diets and changes in eating habits. Recent research suggests that a high intake of refined carbohydrates may also increase the risk of insulin resistance. In addition, diets specifically high in fructose have been shown to contribute to a metabolic disturbance in animal models resulting in weight gain, hyperlipidemia and hypertension.[3]

There is an urgent need for increasing public awareness about the risk associated with high fructose consumption and greater efforts should be made to curb the supplementation of packaged foods with high fructose additives. The prevalence of nonalcoholic steatohepatitis (NASH) is rapidly increasing with the worldwide changes of dietary pattern. Highfructose consumption is a risk factor for NASH.[4,5]The pathogenesis of fructose-induced hypertriglyceridemia and hepatic steatosis is associated with insulin and/or leptin resistance.[6-8] Fructose decreases tyrosine(Tyr) phosphorylation of insulin-induced insulin receptor substrate 1 (IRS1) and inhibits activation of Akt and extracellular signal-regulated kinase 1/2 (ERK1/2)in peripheral tissues of rats.[9,10] Impaired insulin signaling aggravates insulin resistance and suppresses action of insulin on hepatic very-low-density lipoprotein(VLDL) production and triglyceride (TG) secretion, the leading lipid metabolism abnormality in the liver of fructose-fed hamster.[6,7,10] Moreover, fructose-induced hyperleptinemia is observed in animals and humans.[9,10,12]The main action of hepatic leptin is to decrease fatty acid synthesis and to increase fatty oxidation by increasing peroxisome proliferate or-activated receptor α (PPARα) activity, thus reducing hepatic TG synthesis.[13]In fructose-fed rats, over expression of suppressor of cytokine signaling 3 (SOCS3) evoked by high leptin through signal transducer and activator of transcription3 (STAT3) blocks hepatic leptin signaling transduction targeting janus-activated kinase-signal transducer 2(JAK2).[9] ERK1/2 are members of mitogen-activated protein kinase family, which modules PPARa activity. Impaired leptin signaling transduction induced by

fructose decreases hepatic fatty acid oxidation through reduction of ERK1/2 activation and PPARa activity.[8,9]These observations suggest that impairment of insulin or leptin signaling transduction is involved in fructose induced hypertriglyceridemia and hepatic steatosis. Protein-tyrosine phosphatase 1B (PTP1B) is a major negative regulator of insulin signaling.[14] Hepatic PTP1Bexpression is increased in insulin-resistant diabetes and obesity.[15] Mice lacking PTP1B show insulin sensitivity with enhanced phosphorylation of hepatic insulin receptor(IR) and IRS1.[16] The ability of PTP1B to dephosphorylate JAK2 indicates that PTP1B may be involved in the regulation of leptin signaling pathway.[17] PTP1B deficient mice are protected against diet-induced obesity and hypersensitive to leptin.[18] Increased hepatic PTP1B expression is also found in the insulin-resistant state in fructose-fed hamsters.[19,20] These observations indicate that fructose-induced insulin and leptin resistance may be associated with PTP1B abnormality in the liver of animals. However, it is unclear how changes in hepatic insulin and leptin signaling via PTP1B dysregulation can develop lipid metabolism disorder and hepatic steatosis in fructose-fed rats.

In this study NCE-9 protect against experimental hepatic steatosis by improving hyperlipidemia, and insulin resistance. However, the mechanisms underlying its protective actions in fructose induced hepatic steatosis are not completely understood. Inhibition of PTP1B may represent a novel therapeutic approach for the prevention and treatment of hypertriglyceridemia and hepatic steatosis in insulin resistance. NCE-9 prevent hypertriglyceridemia and hepatic steatosisin fructose-fed rats. Rosiglitazone, which exhibits inhibitory effect on PTP1B activity in high-fat rats[25] and has proved useful for treating patients with NASH,[26] was employed as a positive control in this study. Our results provide novel insights into the potential therapeutic mechanisms of NCE-9 on fructose-induced hepatic steatosis associated with insulin resistance.

MATERIALS AND METHOD

Compounds

NCE-9 was synthesized at SNJB's Shriman Sureshdada Jain College of Pharmacy, Nasik, India,were suspended in 0.25% Tween-80+0.5% methyl cellulose solution for in vivo studies.

Animals Experiments

Male Sprague-Dawley rats(4 weeks of age, weighing 200-220g) were used in experiments. Rats were housed with water and food ad-libitum at constant humidity and temperature with a light/dark cycle of 12 hours. All animal use was in compliance Experimental Animal Care issued by the Committee for Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

Fructose induced hypertriglyceridemia and liver steatosis in rats

SD rats (6 weeks) were placed on a chow diet or high fructose diet containing 66% fructose, 12% fat, and 22% protein (Teklad Labs, Madison, WI, USA) for 6 weeks to develop hypertriglyceridemia and liver steatosis. Fructose-fed rats were randomized into six groups (n=8 8) to receive water (vehicle), 3, 10 30, and 100 mg/kg NCE-9, 10 mg/kg Rosiglitazone for an additional 4 weeks, respectively. All drugs were given orally bid. In addition, six animals remained on regular chow to serve as normal control.

Glucose Tolerance Test

At the end of drug treatment, the glucose tolerance test (GTT) were performed, respectively. Rats were weighed and then orally administered with glucose (2 gm/kg) was administered to 12 hr overnight fasted rats and blood samples were collected from the caudal vein by means of a small incision at the end of the tail at 0 (immediately after glucose load), 30, 60 and 120 min after glucose administration. The blood Glucose estimation was done by using Glucometer (Bayer –Contour TS).

Blood and Liver Sample Collection

After GTT all animals were allowed 3 days to recover wounds. Then they were sacrificed after a 16- hour fasting. Blood samples were collected and serum was used for biochemical assays. Liver tissues were dissected quickly on ice and immediately fixed for histological analysis.

Histological Analysis of Liver

Rat livers were removed and immediately fixed for 1 day at room temperature in Carnoy fixative (ethanol: chloroform: acetic acid ¼ 6:3:1) and preserved in 70% ethanol. Renal biopsy specimens were dehydrated with a graded series of alcohol and embedded in paraffin. Specimens were cut in 7- µm-thick section on a rotary microtome and mounted on 3-aminopropyltriethoxysilane (APES)-coated glass slides. Each section was deparaffinized in xylene, rehydrated in decreasing concentrations of alcohol in water, and stained with hematoxylin-eosin reagent(Sigma Chemicals Co., St. Louis, MO). The slide was mounted with neutral balsam.

Serum TG levels levels were determined using common biochemical kits(JianCheng Bioengineering Institute, Nanjing, China).Serum VLDL concentrations, were examined using enzyme-linked Immuno sorbent assay kits (R&D Systems Inc., Minneapolis, MN).

RESULTS AND DISCUSSION

Fructose induced hypertriglyceridemia, characterized by high serum levels of TG, VLDL and

steatosis observed in the livers of fructose fed rats (Figure. 3). NCE-9 treatment dose-dependently attenuated fructose-induced abnormalities (Figure. 3, 4, 5) in rats. Rosiglitazone also had significant effects in this model. Slightsteatosis and inflammation were observed in the livers of fructose-fed rats, which were completely ameliorated by NCE-9 (30 and 100 mg/kg) and Rosiglitazone (Figure.5).

Effect of NCE-9 on glucose tolerance test in SD rat

Oral glucose tolerance test was performed on day 28 of treatment period in high fructose fed rats. On day 28 after chronic treatment, when challenged with an oral bolus of glucose (2 g/kg), NCE-9 (3, 10, 30 and 100 mg/kg, p.o.) treated animals exhibit a reduced AUC glucose (indicating improved tolerance to glucose) compared with vehicle-treated animals (Figure.1, 2).

In the present study, NCE-9 reduced serum levels of TG and VLDL and attenuation of hepatic steatosis in fructose-fed rats. Rosiglitazone has therapeutic efficacy in patients with NASH.[23] In this study, Rosiglitazone also showed beneficial effects on fructose-fed rats. In summary, we firstly demonstrated that NCE-9 prevented fructose-induced hypertriglyceridemia and hepatic steatosis.

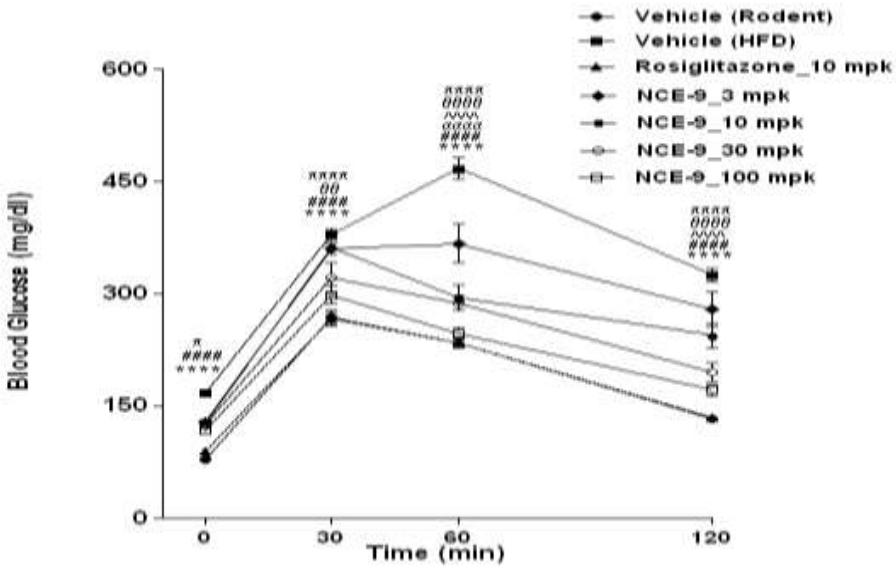

Figure 1.Effect of NCE-9 on blood glucose in OGTT in Fructose fed rats after four weeks of BID treatment

All values are expressed as Blood glucose in mg/dl (Mean ± SEM), n = 6. Vertical lines represent SEM. All data are subjected to Two Way ANOVA followed by Bonferroni'spost test. **** p<0.0001: Vehicle (Rodent diet) vs Vehicle (High Fat diet), ####p<0.0001: Rosiglitazone vs Vehicle (High Fat diet), αααα p<0.0001: NCE-9 (3 mpk) vs Vehicle (High Fat diet), ^^^^p<0.0001: NCE-9 (10 mpk) vs Vehicle (High Fat diet), θθ p<0.01, θθθθ p<0.0001: NCE-9 (30 mpk) vs Vehicle (High Fat diet), π p<0.05, ππππ p<0.0001: NCE-9 (100 mpk) vs Vehicle (High Fat diet).

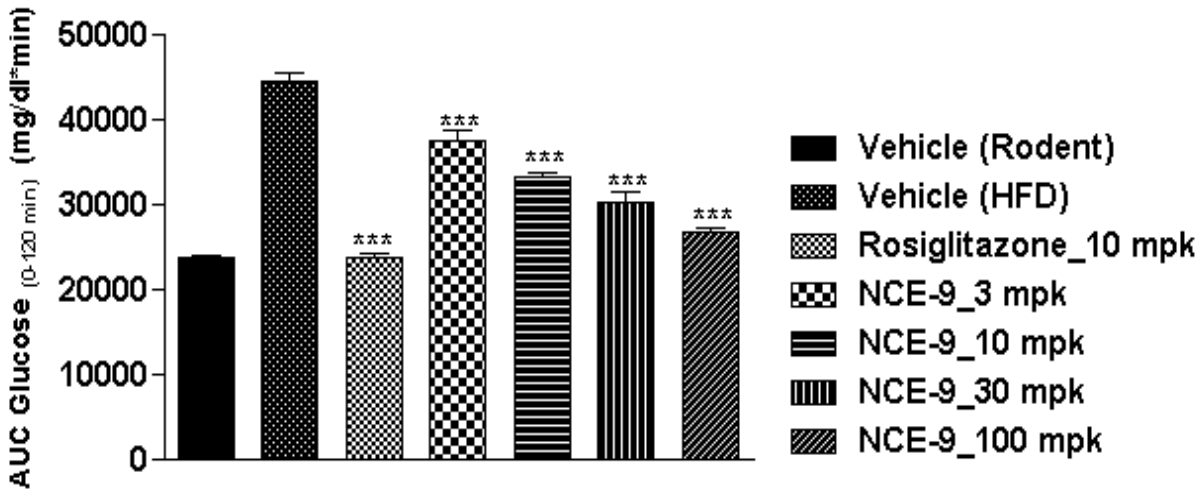

Figure 2. Effect of NCE-9 on AUC Glucose in OGTT in Fructose fed rats after four weeks of BID treatment

All values are expressed as AUC Glucose(0-120 min) in mg/dl*min (Mean ± SEM),n = 6. Vertical lines represent SEM. All data are subjected to One Way ANOVA followed by Dunnett'spost test. *** p<0.001 vs Vehicle

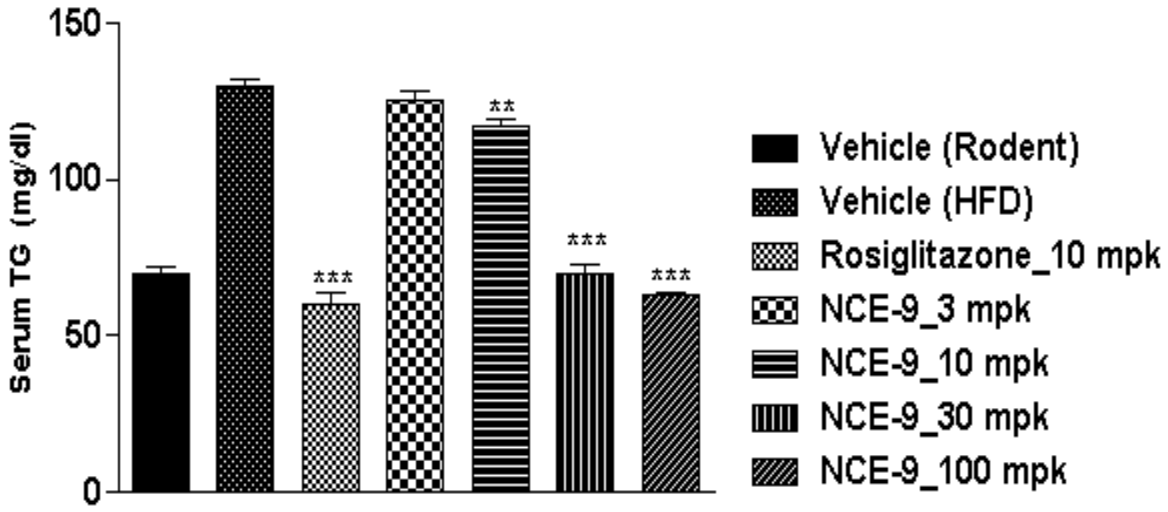

Figure 3. Effect of NCE-9 on Serum TG in Fructose fed rats after four weeks of BID treatment

All values are expressed as Mean ± SEM,n = 6. Vertical lines represent SEM. All data are subjected to One Way ANOVA followed by Dunnett'spost test. ** p<0.01, *** p<0.001 vs Vehicle

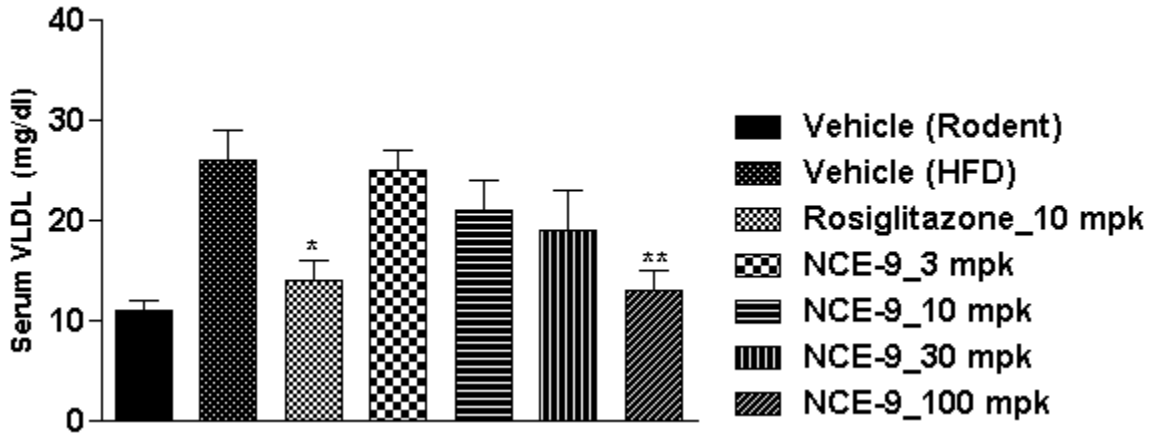

Figure 4. Effect of NCE-9 on Serum VLDL in Fructose fed rats after four weeks of BID treatment

All values are expressed as Mean ± SEM,n = 6. Vertical lines represent SEM. All data are subjected to One Way ANOVA followed by Dunnett'spost test. * $p<0.05$, ** $p<0.01$ vs Vehicle

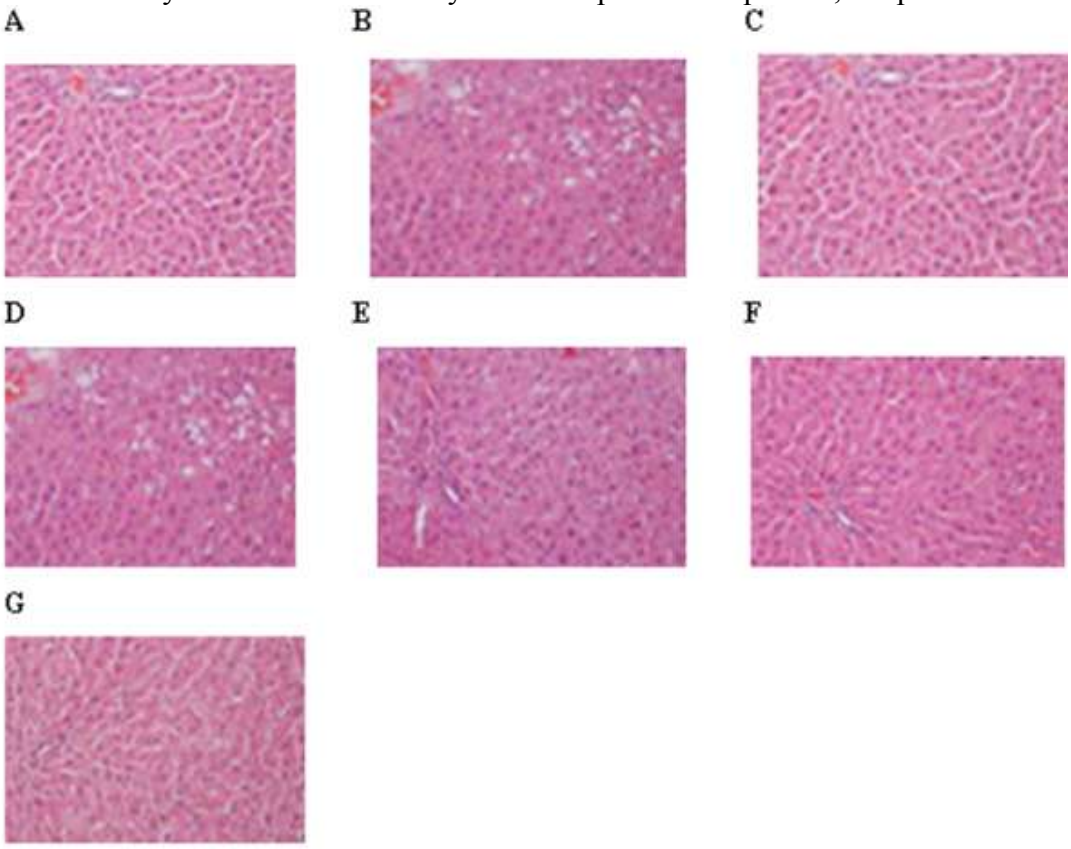

Figure 5.Effects of NCE-9 and rosiglitazone on hepatic steatosis in fructose-fed rats on Liver sections from these groups at a magnification of 20X

A: Vehicle (Rodent diet); B: Vehicle (High Fat diet); C: Rosiglitazone; D: NCE-9 (3 mpk); E: NCE-9 (10 mpk);F: NCE-9 (30 mpk); G: NCE-9 (100 mpk)

CONCLUSION

These findings suggest a protective effect of NCE-9 against the metabolic abnormalities induced by high fructose diet. However, further studies should be carried out to explore beneficial use of NCE-9 as PTP1B inhibitors in human subjects suffering from diabetes and obesity.

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