Partial PPAR γ agonist NS-1 improves insulin resistant, vascular function and lowers blood pressure in fructose fed rats.

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Abstract— Thiazolidinedione's, PPARγ ligands used clinically to treat type-II diabetes, have been reported to lower blood pressure and provide other cardiovascular benefits. The aim of the present study was to observe the effect of NS-1 (a selective partial activator of PPARγ) on cardiovascular and metabolic parameters in high fructose fed rats.

Effect of NS-1 on the expression and secretion of adiponectin was performed in vitro in 3T3-L1 adipocytes. In addition, cardio-vascular (Blood pressure and vascular response), metabolic (Glucose tolerance test), anti-oxidant (Superoxide dismutase and catalase) and lipid peroxidation (Malondialdehyde) effects of NS-1 (1, 3 and 10 mg/kg, p.o.) were determined in high fructose fed Sprague–Dawley rats.

NS-1 showed dose dependent increased in expression and secretion of adiponectin in 3T3-L1 adipocytes. NS-1 (3 and 10 mg/kg, p.o.) significantly reduced blood pressure, glucose intolerance and oxidative stress in high fructose fed rats. Furthermore, NS-1 (3 and 10 mg/kg, p.o.) improved vascular dysfunction in addition to increase in eNOS protein and adiponectin level. NS-1 administration restores cardio-vascular and metabolic impairment induced by high fructose diet in Sprague–Dawley rats.

Key words: NS-1, Peroxisome proliferator-activated receptors (PPARs), Insulin resistant, Blood pressure.

1 Introduction

Peroxisome proliferator activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and bind to DNA as a heterodimer with retinoid x receptor to regulate transcription (1, 2). PPARs have 3 isoforms, α, β/δ, and γ, which bind to sequence-specific target elements as a liganded hetero-dimer with the retinoid x receptor in the promoter region of target genes controlling nearly every step in cellular fatty acid uptake, utilization, oxidation, storage pathways, cell growth and migration, oxidative stress and inflammation in the cardiovascular system (3-5).

Cardiovascular disease is one of the leading causes of death in the western world and diabetes mellitus has been identified as a primary risk factor, due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators (6, 7).

PPARγ and its ligands or agonists have a definite blood pressure lowering action in patients or animal models of diabetes/metabolic syndrome. These blood pressure-reducing properties, which are not present with metformin or sulfonylureas, are particularly important when viewed in conjunction with hypoglycemic effects. A significant proportion of patients with type 2 diabetes mellitus and blood pressure mildly above target range might be treated for both processes with a single drug (8).

These advantages have initiated the search for some novel potential partial PPARγ agonist with lesser side effect. In an effort to search for novel PPARγ agonists; we screened a library of various structurally diverse synthetic compounds. Among active compounds identified, a compound with indene structure was chosen based on the novelty and ease of derivative synthesis and chemical modification of this molecule lead to the NS-1 as a lead compound for novel PPARγ agonists (Fig. 1). NS-1 chemically known as (5Z)-5-[4-hydroxy-3-methoxy-phenyl) methylene] thiazolidine-2, 4-dione), detailed synthesis and QSAR related to NS-1 is already published (9).

Our previous study demonstrates that NS-1 is functionally active as a selective partial PPARγ agonist shown by transactivation assay and having week adipogenic activity. Chronic treatment with a novel partial PPARγ agonist blunted the development of diabetes in diet induced obese mice by improving the glucose tolerance and insulin sensitivity without demonstrating the adverse effects on body weight gain typically seen with PPARγ agonists (10).

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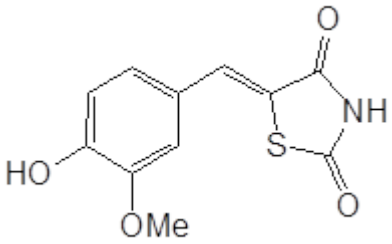


Fig 1 Chemical structure of NS-1 (5Z)-5-[4-hydroxy-3-methoxy-phenyl) methylene] thiazolidine-2, 4-dione).

In the present study we characterized the effects of oral administration of this compound in high fructose diet induced hypertension, hypertriglyceridemia and insulin resistance in Sprague–Dawley rats.

2. MATERIALS AND METHODS

2.1 Formulation of NS-1

NS-1 and pioglitazone were synthesized at Poona College of pharmacy, Pune, India. The compounds were suspended in 0.5% Tween-80 + 0.5 % Carboxy methyl cellulose solution for in vivo studies.

2.2 Effect of NS-1 on the expression and secretion of adiponectin in 3T3-L1 adipocytes

Mouse 3T3-L1 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS) and differentiated with DMEM supplemented with 5 mg/ml of insulin, 0.5 mmol/l 1-methyl-3-isobutyl-xanthin, and 1 mmol/l dexamethazone 2 days after reaching confluence. On day 7, the indicated concentrations of NS-1 or Pioglitazone were added to the media for 24 h. Adiponectin mRNA levels were measured by Northern blotting same as reported elsewhere (11). An aliquot of the media from 24 h after stimulation was subjected to western blotting to detect the amount of adiponectin that was secreted (11).

2.3 In Vivo pharmacokinetic profile of NS-1

Sprague Dawely rats (adult males, 250-300 g) fasted for 6 h were administerd NS-1 at 10 mg/kg for oral and 1 mg/kg for intravenous pharmacokinetic profile. Blood samples were collected at different time interval by retroorbital puncture and separated plasma was stored at -80 ^{0}C until used. Compound concentrations in plasma were determined by HPLC analysis and the pharmacokinetic parameters were calculated by a non-compartmental method with WinNolin professional Version 4.1.

2.4 Fructose feeding protocol

Male Sprague Dawely rat were purchased from National Toxicology Center, Pune, India. Animals were housed in clean environment under 12:12 light: dark cycle at a temperature of 25 ± 2 ^{0}C and relative humidity of 55 ± 5%. Food and water were available ad libitum. All experimental procedures using animals were performed under the guidelines of our institutional animal ethical Committee. Sprague Dawely 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 16 weeks to develop hypertension, hypertriglyceridemia and insulin resistance. At the end of sixteen weeks, blood pressure was measured by tail cuff method and rats with systolic blood pressure higher than 150 mmHg and Blood glucose level higher than 160 mg/dl were selected, randomized into groups and used for the study. Study was performed in two groups of animals, one group (n=10) is used for metabolic paremetres and another group (n=10) is used for cardiovascular parameter. NS-1 and standard compound were given orally twice a day for next 4 weeks.

2.5 Blood pressure measurement

Rats were removed from the animal room and taken to the laboratory at 0900; they were allowed free access to diet and water and kept in a quiet area before the blood pressure was measured at 1200. The tail-cuff method was used to measure the blood pressure with the help of Panlab NIBP system and rats were acclimatized for 3 days before blood pressure measurement (12). Systolic, Diastolic and Mean arterial Blood pressure was recorded every week and the mean of thee consecutive readings were used as the measurement of the blood pressure of each rat for that day.

2.6 Preparation of aortic rings.

The thoracic aorta of rats was isolated immediately after decapitation and carefully cleaned of fat and connective tissues. The aorta was cut into rings of 3 mm width. Extreme care was taken not to stretch or damage the luminal surface of the aorta to ensure the integrity of endothelium. In some rings endothelium was denuded by gently rubbing the aortic rings with forceps. Aortic rings were suspended between two 'S' shaped platinum loops in jacketed organ bath containing 10 ml Krebs bicarbonate solution (pH 7.4) maintained at 37^{o}F and continuously aerated with 95% oxygen and 5% carbon dioxide. The composition of the Krebs solution (mM) was NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 22.0, and glucose 11.0. The rings were connected to isometric force displacement transducer connected to Data Acquisition Systems – Power Lab (AD instruments). The rings were maintained under tension of 2 g and equilibrated for 90 min before initiating experimental protocol. During this period, the Krebs solution was changed at every 15 min interval. After the equilibration period, rings were maximally contracted with Phenylephrine (PE, 1 μM) to test their contractile capacity. The presence of functional endothelium was assessed by the ability of acetylcholine (0.1 μM) to induce more than 60% of relaxation of rings precontracted sub maximally with phenylephrine. Aortic

rings were considered denuded when there was less than 10 % relaxation to Ach.

2.7 Effect of phenylephrine and acetylcholine on aortic rings obtained from NS-1 treated rats

After 4 weeks of treatment aortic rings of respective group were isolated and mounted in organ bath as described above. Concentration–response curves to increasing concentrations of phenylephrine (300 pM–3 μM) were performed in rings with intact endothelium. Concentration–response curve of phenylephrine with the presence and absence of 100 μM of L-NAME was also recorded. Indomethacin (10 μM) was added to prevent the involvement of prostaglandins. Endothelium mediated relaxation was measured as a concentration–response curve to acetylcholine (1 nM–10 μM) in rings precontracted with phenylephrine in presence and absence of 100 μM of L-NAME (13).

2.8 Western blot for eNOS protein

After 4 weeks of treatment, aortic tissues of different group were homogenized in 25 mmol/L of Tris-HCl buffer (pH 6.8) with (in mmol/L) sodium dodecylsulfate (SDS) 100, ethylenediaminetetraacetic acid, dithiotheitol and phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 14000 g for 120 minutes at 4°C, and the supernatant was boiled for 10 minutes. Tissue lysates (80 μg for each sample) were separated on 7.5% SDS-polyacrylamide gels, and transferred to a polyvinyl difluoride membrane (Millipore Corp.). The membranes were blocked with 10% skim milk in Tris-buffered saline pH 7.4, containing 0.1% Tween 20 for 6 hours at room temperature, and incubated overnight at 4°C with a 1:500 dilution of mouse monoclonal antibody to eNOS (Transduction Laboratories). The membranes were incubated for 1 hour with 1:1000 dilution of antimouse secondary antibody conjugated to horseradish peroxidase (Amersham). Bound antibody was detected by enhanced chemiluminescence (ECL kit, Amersham) on XAR film (Kodak). β-actin (monoclonal anti β-actin antibody, Sigma) was used for all membranes as an internal control, and signals on Western blots were quantified by densitometry and normalized relative to the β-actin signal by use of an image analysis software program (14).

2.9 Measurement of antioxidant and lipid peroxidation

After 4 weeks of treatment, animals were sacrificed to isolate and weigh the aortas. The levels of malondialdehyde (MDA) and the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were determined in the aorta tissue by using different cell biolab assay kit.

2.10 In Vivo efficacy of NS-1 on metabolic parameters.

High Fructose fed rats were orally gavaged with the NS-1 (1, 3 and 10 mg/kg, p.o.) and pioglitazone (10 mg/kg, p.o.) for 28 days. Animals were randomized based on pretreatment fed blood glucose levels and body weights. An oral glucose tolerance test (OGTT) was performed at day 22. Ad lib fed blood glucose was observed weekly. Animals were sacrificed at day 28 for plasma and tissue collection. Biochemical parameter were analysed from day 28 plasma samples.In OGTT assay, an oral dose of vehicle or compounds were given in 12 h fasted rats after fasting blood glucose (t = -30 min) measurement. The Animals were then gavaged with an oral bolus of glucose (2g/kg) after baseline blood glucose measurement. Subsequent blood glucose measured at 15, 30, 60, 120 and 180 min.

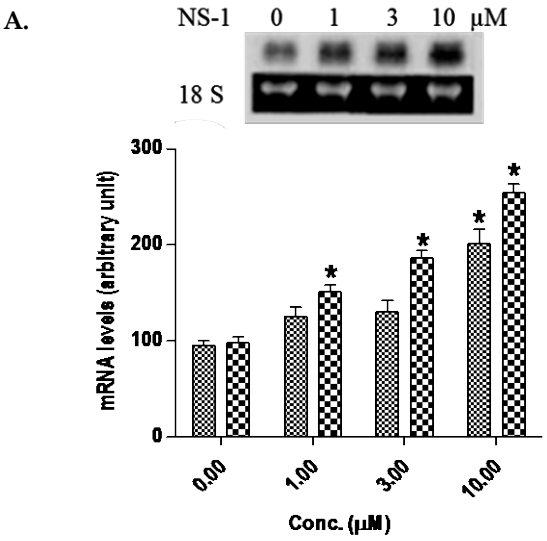
2.11 Biochemical assay

Total plasma cholesterol, triglyceride and Non-esterified fatty acid (NEFA) levels were measured from frozen plasma using the Hitachi 717 clinical chemistry analyzer. Blood glucose levels were measured immediately following blood collection on a glucometer (Accuchek performa). Reagents for cholesterol and triglyceride measurements were purchased from Merck, USA). Reagents for NEFA measurement were obtained from Randox. Insulin levels were measured by using Mercodia ELISA assay kit and Adeponectin levels were measured by Biovision kit.

3. RESULTS

3.1 Inducible effects of NS-1 on the expression and secretion of adiponectin in 3T3-L1 adipocytes

We investigated the effect of NS-1 on the regulation of adiponectin gene expression in 3T3-L1 adipocytes. Incubation with NS-1 enhanced adiponectin mRNA expression and adiponectin secretion into the media in a dose-dependent fashion (Fig. 2A, B). NS-1 at 10 μM showed significant increased in adiponectin mRNA expression and adiponectin secretion in 3T3-L1 adipocytes which is comparable with pioglitazone.



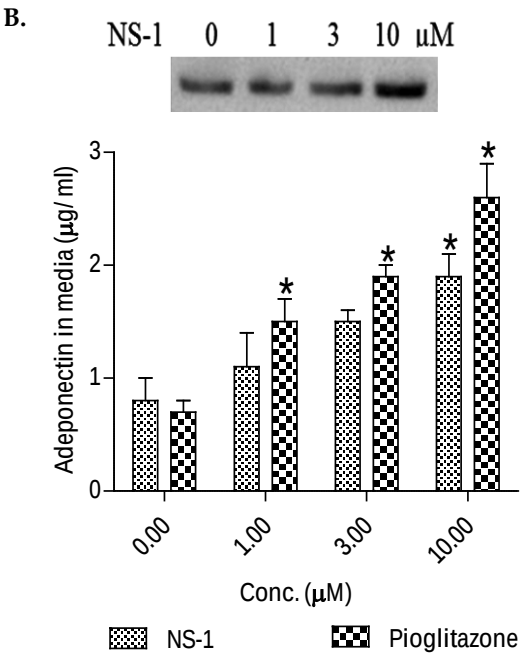


Fig 2 Effects of NS-1 on expression and secretion of adiponectin in 3T3-L1 adipocytes. Dose dependent effect of NS-1 on the adiponectin mRNA level (A) and the secreted amount in media (B) is shown. After differentiation-induction on day 7, 3T3-L1 cells were treated with the indicated concentrations of 0, 1, 3, and 10 µM for 24 h. Five micrograms of total RNA was subjected to Northern blotting and quantified and normalized relative to the 18-s rRNA signal. All mRNAs are plotted as percentage change relative to mRNA level by 0 µM NS-1 treatment. The amount of adiponectin secreted into media was measured by quantitative Western blotting. The inset shows a representative picture of Northern and Western blotting that was quantified. Values are expressed as mean ± SEM. (n=6).

3.2. In Vivo pharmacokinetic profile of NS-1

Sprague–Dawley rats received an oral dose of 10 mg/kg of NS-1 to observe the in vivo pharmacokinetic profiles. As indicated in Table 1, NS-1 shows Cmax at 1.74 µM and T max was 0.3 h. Absolute bioavailability of NS-1 was 56%, showing a good pharmacokinetic profile.

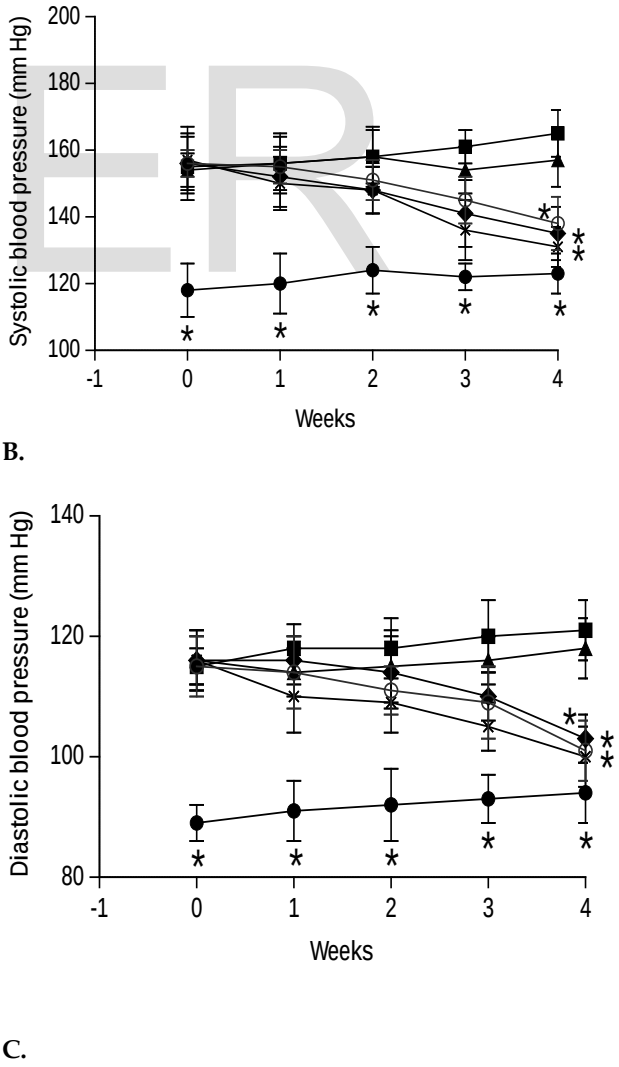
3.3 Effect of NS-1 on blood pressure

There was a significant increase in systolic, diastolic and mean arterial blood pressure in high fructose diet fed animals as compared to normal diet fed animals. NS-1 (3 and 10 mg/kg/day, p.o.) treatment for 4 weeks significantly reduced systolic, diastolic and mean arterial blood pressure as compared to vehicle group. Pioglitazone (10 mg/kg/day, p.o.) treatment for 4 weeks significantly reduced systolic, diastolic and mean blood pressure as compared to vehicle group (Fig. 3).

Parameters	I.V. (1 mg/kg)	P.O. (10 mg/kg)
Cmax (µM)	7.29	1.74
Tmax (h)	0.08	0.3
AUC (0-24h) (µM.h)	3.53	1.6
Vss (L/Kg)	1.2	NA
CL (ml/min/Kg)	39.22	NA
$T_{1/2}$ (h)	0.49	NA
F%	NA	56

Pharmacokinetic parameters of NS-1 after i.v. (1 mg/kg) and p.o. (10 mg/kg) administration to male SD rats (mean ± SEM, N = 6 animals/route of administration).

Table 1 Pharmacokinetic parameters of NS-1



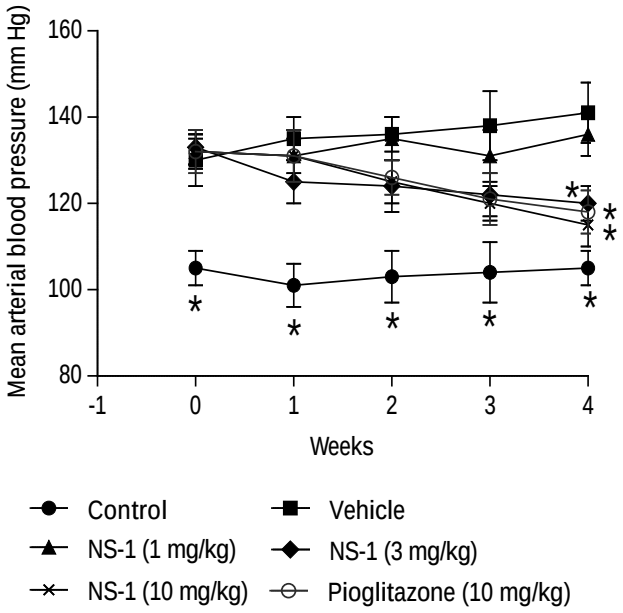


Fig 3 Effect of NS-1 treatment for 4 weeks on systolic (A), diastolic (B) and mean arterial blood pressure (C) in high fructose fed Sprague dawely rats. Values are expressed as mean ± SEM. * P< 0.05, compared to vehicle treated group. (n=10).

3.4 Contractile response to phenylephrine on aorta obtained from NS-1 treated rats

Cumulative addition of phenylephrine (300 pM–3 μM) resulted in concentration dependent contraction of aorta in all the groups (Figure 4) . There was a significant ($p<0.05$) increase in maximal response (Emax) of phenylephrine in aorta obtained from vehicle group as compared to normal group. pD_2 value of Phenylephrine in aorta obtained from vehicle group was significantly ($p<0.05$) higher as compared to normal group. Contractile response of phenylephrine in aorta obtained from NS-1 group was decreased in dose dependent manner as compared to vehicle group. Maximal response (Emax) and pD_2 value of phenylephrine in aorta obtained from NS-1 group (3 and 10 mg/kg, p.o.) was significantly ($P<0.05$) decreased as compared to vehicle group. Maximal response (Emax) and pD_2 value of Phenylephrine in aorta obtained from pioglitazone group (10 mg/kg, p.o.) was significantly ($P<0.05$) decreased as compared to vehicle group (Table 2). There was no significant change in pD_2 value of phenylephrine in aortic rings of vehicle group due to the presence of L-NAME. (Data not shown).

3.5 Relaxation response to acetylcholine on aorta obtained from NS-1 treated rats

Addition of acetylcholine to all aortic rings with intact endothelium resulted in concentration dependent relaxation of rings that were precontracted with phenylephrine ((Figure 5). Acetylcholine induced relaxation in aorta obtained from vehicle group was significantly ($p<0.05$) lower as compared to normal group. pD_2 value of acetylcholine in vehicle group was significantly ($p<0.05$) lower as compared to normal group. pD_2 value of acetylcholine in aorta obtained from NS-1 (3 and 10 mg/kg, p.o.) and Pioglitazone (10 mg/kg, p.o.) treated group was significantly ($p<0.05$) higher as compared to vehicle group (Table 3). pD_2 value and % relaxation induced by acetylcholine in aortic rings obtained from NS-1 treatment group (1, 3 and 10 mg/kg, p.o.) and pioglitazone (10 mg/kg, p.o.) was not significantly increased as compared to vehicle group due to the presence of L-NAME (Data not shown).

3.6 Effect of NS-1 on eNOS protein.

Representative Western blots conducted on proteins from thoracic aortas after 4 week treatment with NS-1 are illustrated in Figure 6, respectively. The accompanying histograms illustrate the mean aortic eNOS protein levels (arbitrary units of optical density) for each group (n=10 per group). Aortic eNOS protein was decreased in vehicle group as compared to normal group. Aortic eNOS protein was increased dose dependently after 4 week treatment of NS-1 (1, 3 and 10 mg/kg, p.o.). Aortic eNOS protein was also increased in pioglitazone (10 mg/kg, p.o.) treated animals.

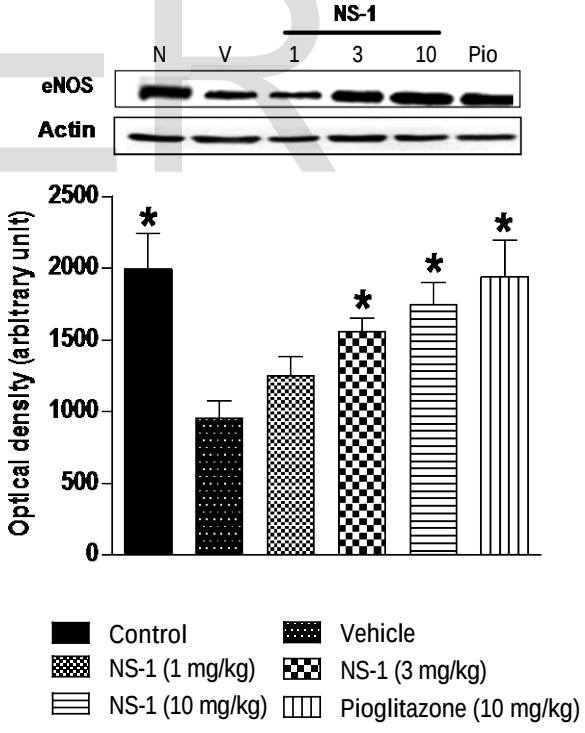


Fig 6 Representative Western blots of eNOS carried out on proteins from thoracic aortas after 4 week of treatment NS-1. Histograms illustrate the mean aortic eNOS protein levels (arbitrary units of optical density) for each group. Values are expressed as mean ± SEM. * P<0.05, compared to vehicle group. (n=10).

3.7 Effect of NS-1 on superoxide dismutase, catalase and lipid peroxidation

Oxidative stress was significantly ($p<0.05$) increased in aorta of vehicle group as compared to normal group (Table 4). Superoxide dismutase and Catalase were significantly decreased while lipid peroxidation was significantly increased in vehicle group as compared with normal group. NS-1 (3 and 10 mg/kg, p.o.) treatment significantly ($p<0.05$) increased levels of endogenous antioxidants (SOD and CAT) in aorta as compared to vehicle group (Table 4). Moreover lipid peroxidation was significantly ($p<0.05$) decreased in aorta of NS-1 (3 and 10 mg/kg, p.o.) as compared to vehicle group (Table 4). Pioglitazone (10 mg/kg, p.o.) treatment significantly ($p<0.05$) increased levels of endogenous antioxidants (SOD and CAT) in aorta as compared to vehicle group (Table 4). Moreover lipid peroxidation was significantly ($p<0.05$) decreased in aorta of pioglitazone (10 mg/kg, p.o.) as compared to vehicle group (Table 4).

3.8 Effect of NS-1 on metabolic parameters and body weight

NS-1 (3 and 10 mg/kg, p.o.) treatment results in dose dependent lowering of fed blood glucose from day 7 to day 28 (Fig ure 7). NS-1 (3 and 10 mg/kg, p.o.) showed significant decrease in fasted plasma glucose, insulin, triglyceride, total cholesterol, NEFA and adiponectin levels after 4 week of treatment (Table 5). Amelioration of hyperglycemia in the presence of reduced plasma insulin levels suggests that insulin sensitivity has been improved in NS-1-treated rats. Pioglitazone at 10 mg/kg, p.o. showed significant increase in body weight and feed intake while NS-1-treated rats did not showed significant change in body weight and feed intake in comparison with the vehicle-treated rats (Table 5). Pioglitazone at 10 mg/kg, p.o. showed significant increase in omental fat while NS-1-treated rats did not showed significant change in omental fat in comparison with the vehicle-treated rats which correlates with body weight changes. Oral glucose tolerance test was performed on day 22 of treatment period in high fructose fed rats. On day 22 after chronic treatment, when challenged with an oral bolus of glucose (2 g/kg), NS-1 (3 and 10 mg/kg, p.o.) treated animals exhibit a reduced AUC glucose (indicating improved tolerance to glucose) compared with vehicle-treated animals (Figure 8A, B).

4. DISCUSSION

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-activated transcription factor of the nuclear hormone receptor superfamily. Increasing evidence suggests that PPAR γ is involved in the regulation of vascular function and blood pressure in addition to its well-recognized role in metabolism. Thiazolidinediones, PPAR γ agonists, lower blood pressure and have protective vascular effects though largely unknown mechanisms (15). In the present study, we examined the effect of NS-1 on metabolic parameters induced by high fructose diet. Fructose administration in the diet was able to induce systemic hypertension, insulin resistant and hypertriglyceridemia (16). These data thus demonstrate a direct relationship between increasing fructose consumption and worsening features of the metabolic syndrome.

There are several important findings. First, NS-1 treatment reduces blood pressure and improves the thoracic aorta response to Acetylcholine and phenylephrine. Second, the mechanisms of this improvement appear to involve changes in NO mediated signalling, increase in eNOS protein and decreased in oxidative stress in thoracic aorta by NS-1. Third, NS-1 treatment improves insulin resistant and also increases adeponectin level.

The results from the isolated aortic studies demonstrated that aorta from 16-week fed high fructose fed rats is more responsive to phenylephrine and relaxation response to Acetylcholine was significantly decreased than chow fed animals. Similar results showing the increase vascular responsiveness to phenylephrine and decreased Acetylcholine induced relaxation in high fructose fed rats have been reported in previous studies (17). Moreover the levels of endogenous antioxidants (Superoxide dismutase and catalase) were significantly reduced and lipid peroxidation significantly increased in high fructose fed rats group showing increased oxidative stress. Decrease contractility could be due to deficient endothelial activity, enhancement of oxidative stress due to excessive production of oxygen-free radicals and decreased antioxidant defence systems (18).

It is a well-known fact that endothelium-dependent relaxation response to agonists such as acetylcholine is impaired in diabetic rat aorta (19). There are two possible mechanisms for reductions in acetylcholine-induced relaxation, first nitric oxide (NO)-dependent vasodilatation, i.e. a decrease in NO release (or production) from the endothelium and a decreased reactivity of vascular smooth muscle to NO in diabetic animals. Another possible mechanism of reduced responses to acetylcholine in diabetic animals is that oxidative degradation and inactivation of NO may be increased in vessels of such rats. Several studies have indicated the increased production of superoxide anions in vessels of diabetic animals. Further it is suggested that this active form of oxygen can inactivate NO to attenuate NO-dependent vasodilatory response in the diabetic rabbit aorta (20,21) . High fructose fed rats showed increased oxidative stress along with decrease vascular contractility and decreased acetylcholine induced relaxation. Therefore, the oxidative stress in these animals might be responsible for decreased contractility together with deficient endothelial function.

Administration of NS-1 for 4 weeks restored the elevated blood pressure, reduced the enhanced contractility to PE and acetylcholine induced relaxation was restored. In NS-1 treated high fructose fed rats there was an increase in acetylcholine induced relaxation which may be due to involvement of NO pathway since the relaxation was blocked with the presence of

L-NAME. Further tone related NS-1 treatment also increases eNOS protein in thoracic aorta of rats fed with high fructose diet.

PPARγ agonists ameliorate insulin resistance and dyslipidemia in type 2 diabetic patients. Adiponectin possesses insulin sensitizing properties, and predicts insulin sensitivity of both glucose and lipid metabolism (22). PPARγ agonists increase insulin sensitivity and circulating adiponectin (23,24). The current study demonstrated that NS-1 upregulated adiponectin level in high fructose fed rats. In addition, NS-1 treatment increases the adiponectin level and expression in dose dependent manner in 3T3-L1 adipocytes. Thiazolidinedione insulin sensitizers are well known as PPARγ agonists and PPARγ activation appears to contribute to the hypoglycemic effect in vivo (25). In the present study, the antihyperglycemic activity of NS-1 was tested in an established animal model of type 2 diabetes, i.e., using high fructose fed rats. The in vivo data demonstrate that NS-1 is a potent and efficacious antidiabetic agent in high frustose fed rats. After chronic treatment with NS-1 lowers fasted blood glucose, insulin, triglycerides, cholesterol, NEFA levels comparable with rats fed on normal diet. In addition, glucose clearance was significantly increased in rats treated with NS-1 and the finding was confirmed by AUC analysis.

PPARγ activation is well known to increase body weight and feed intake. Two major mechanisms of PPARγ-mediated body weight gain are increase of adipocyte differentiation and fluid retention (26). Pioglitazone (10 mg/kg, p.o.) showed increase in body weight and feed intake after 4 week of treatment in high frustose fed rats. While NS-1 (10 mg/kg, p.o.) did not showed significant change in body weight and feed intake that also well correlated with our previous adipocyte differentiation and adipogenesis studies where NS-1 showed week adipocyte differentioan and adipogenic porperties in compare with pioglitazone (10).

In summary, these functional, biochemical, and molecular findings suggest a protective effect of NS-1 against the cardiovascular and metabolic abnormalities induced by high fructose diet. However, further studies should be carried out to explore beneficial use of partial PPARγ agonists in human subjects suffering from metabolic syndrome.

5. ACKNOWLEDGMENT

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