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1-trifluoromethoxyphenyl-3(1-propionylpiperidin-4-yl) urea (TPPU), a soluble epoxide hydrolase inhibitor attenuates high fat diet-induced cardiovascular and metabolic disorders in rats

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Abstract. – OBJECTIVE: Obesity was induced in rats by feeding on a high fat diet (HFD), 60% w/w cholesterol, 20% w/w carbohydrates, and 20% w/w proteins for two months.

MATERIALS AND METHODS: Animals were fed on a HFD and treated concurrently with a single daily dose of vehicle or TPPU (2 mg/kg p.o) for two months. Body weights, blood pressure, and biochemical investigations of all animals were registered at 0, 1, and 2 months of the experimental period.

RESULTS: Vehicle-treated rats fed on a HFD had a considerable increase in body weight compared to age-matched control animals fed on a regular diet (regular diet; 311.40 ±9.60 vs. HFD; 446 ± 12.67). The body weight of rats fed on a HFD and concurrently treated with 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4yl) urea (TPPU; 2 mg/kg p.o) daily for two months was significantly decreased (p<0.01). A significant (p<0.01) increase in the systolic blood pressure of animals and vascular dysfunction with blunted relaxant response to acetylcholine and sodium nitroprusside was evident in vehicle-treated animals fed on a HFD compared to control rats fed on a regular diet. These HFD-induced disorders were markedly attenuated in animals fed on a HFD and treated concurrently with a single daily dose of TPPU (2 mg/kg p.o). HFD diet-induced deleterious metabolic changes were prevented with concurrent administration of TPPU (2 mg/kg p.o). TPPU treatment decreased the HDF-induced increase in plasma creatinine levels (p<0.001) in rats. The adiponectin levels were decreased (p<0.001) in vehicle-treated rats fed on HFD for two months compared to control rats fed on a normal diet (p<0.001). Adiponectin levels were significantly (p<0.001) increased in rats fed on HFD and treated concurrently with TPPU (2 mg/kg p.o). HFD diet caused a marked increase in plasma leptin levels of animals which were significantly decreased in animals fed on a HFD and treated concurrently with TPPU for two months. Obese animals exhibited increased levels of plasma insulin compared to control animals fed on a regular diet which were significantly suppressed (p<0.001) by TPPU treatment. In the current investigation, TPPU treatment had a favorable impact on the levels of other metabolic parameters such as plasma cholesterol, triglycerides (TGs), low density lipoproteins (LDLs), and high density lipoproteins (HDL). HFD caused a profound increase in the serum liver enzymes, the effect was reversed by treatment of animals with TPPU (2 mg/kg p.o).

CONCLUSIONS: The findings of our current study indicate the promising therapeutic potential of TPPU as a new drug candidate to manage obesity-induced cardiovascular and metabolic disorders. Soluble epoxide hydrolase inhibitors such as TPPU could prevent HFD-induced obesity and related cardiovascular and metabolic complications.

Key Words:
TPPU, High fat diet, Obesity, Cardiovascular dysfunctions, Metabolic disorders.

Introduction

Obesity is a major risk factor for the development of diabetes and cardiovascular diseases. Substantial evidence has demonstrated that obesity is a major risk factor for cardiovascular diseases, including hypertension, vascular endothelium

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dysfunction, dyslipidemia, insulin resistance, and diabetes mellitus[1,2]. Obesity and associated metabolic syndrome are characterized by increased oxidative stress along with inhibition of the cardio-protective adiponectin[3]. Earlier studies[4-6] in animal models of obesity have revealed that HFD led to hyperglycemia, insulin resistance, dyslipidemia, vascular dysfunction, and hypertension. It has been reported that obese individuals and rats fed on a HFD showed a significant reduction in epoxyeicosatrienoic acids (EETs) production[7,8]. HFD-induced obesity in rats showed reduced expression of cytochrome p-450 as well as a reduction in EETs levels[7,9]. sEH activity is higher in the fat pads of mice fed a western diet (fat-rich) than in normal feed pellet-fed mice[10]. Increased expression of soluble epoxide hydrolase (sEH), the EETs metabolizing enzyme, may contribute to obesity and diabetes-associated metabolic and cardiovascular complications[11]. Increasing or stabilizing EETs by sEH inhibitors (sEHIs) such as TPPU and AUDA improve renal function and reduce blood pressure in obese rats[12,13]. Moreover, EETs exert beneficial effects on obesity-associated diseases[2]. The inhibition of sEH results in increased EETs[14], and sEHIs, such as TPPU, have been shown to protect against a variety of diseases, including hypertension-induced end-organ damage, atherosclerosis, aortic aneurysm, cardiac hypertrophy, arrhythmia, stroke, heart attack, pulmonary hypertension, chronic obstructive pulmonary disease, and renal failure in various animal models[15,16]. sEHIs improve diet-induced obesity-related hypertension and insulin resistance[17]. Based on these studies and to substantiate the beneficial effects of EETs in obesity, we postulated that the increased endogenous levels of EETs by inhibiting their metabolism using TPPU can prevent or attenuate the HFD diet-induced cardiovascular and metabolic complications in obese rats. Effects of TPPU were evaluated on high-fat diet-induced obesity and the related cardiovascular and metabolic complications in rats.

Materials and Methods

Materials

High fat diet (60% w/w cholesterol, 20% w/w carbohydrates, and 20% w/w proteins) was purchased from Research Diets, Inc. (New Brunswick, NJ, USA), phenylephrine (PE), acetylcholine (Ach), phenylephrine, and sodium nitroprusside were procured from Sigma-Aldrich (St. Louis, MO, USA). TPPU was purchased from Synthia Laboratories (Davis, CA, USA). Physiological salt solutions were prepared with potassium chloride (Sigma-Aldrich, St. Louis, MO, USA), sodium bicarbonate, magnesium sulfate, potassium dihydrogen phosphate, glucose, sodium chloride, and calcium chloride (E. Merck, Darmstadt, Germany). The chemicals used in the studies were of analytical grades, and their solutions were prepared in distilled water.

Animals

Sprague-Dawley (SD) rats (150-200 g) of either sex were housed at 23-25°C in the Animal House facility of College of Medicine, King Saud University Riyadh, Kingdom of Saudi Arabia. Rats (n=12), were kept separately in cages (3 in each cage) with free access to water and food. The temperature of the room was maintained at 23±2°C with 12 h dark/light cycles. All studies followed the guidelines of the National Committee for Ethics and Care of Experimental Animals, King Saud University, Riyadh. The study was conducted in accordance with the Basic and Clinical Pharmacology and Toxicology policy for experimental and clinical studies[18].

Methods

Induction of Obesity

Obesity was induced by feeding rats on high fat diet (60% w/w cholesterol, 20% w/w carbohydrates, and 20% w/w proteins) for two months. Animals were administered orally for 2 months' vehicle or TPPU (2 mg/kg p.o).

Experimental design – Group-I Control: Animals (n=12) were provided tap water and standard rodent diet for two months. Group-II: Animals (n=12) were treated with vehicle (0.1% Tween 80 in water) and provided tap water and HFD for two months.

Group-III: Animals (n=12) were treated concurrently with a single daily dose of TPPU (2 mg/kg p.o) and provided tap water and HFD for two months. The selected dose of TPPU (2 mg/kg p.o) was based on the effects of the compound in our initial experiments and justification described elsewhere[16].

Non Invasive Blood Pressure Measurement

Blood pressure (BP) was measured by the non-invasive tail-cuff technique (Model MK-

2000, Murom chi Kikai Co., Ltd., Tokyo, Japan). Systolic blood pressure in control and treated rats were monitored at 0, 1, 2 months. The rats were acclimatized and trained for the measurement of blood pressure before starting the experiments. Systolic blood pressure was measured by taking an average of 3 readings of each conscious animal.

Vascular Reactivity Studies

At the end of the study, rats were euthanized, and the thoracic aorta was isolated. Measurements of isometric tone in rat aorta rings were conducted according to the method as described elsewhere[19] with some modifications. Briefly, the aorta was isolated, cleaned off fat and connective tissue, placed in cold Krebs's solution. Aorta was sectioned into 3-mm-long rings and mounted into four-chamber wire myograph, Danish MyoTechnology (DMT), Denmark organ bath system – The vessels were allowed to achieve equilibrium for at least 45 min. The maximum constriction of the vessels was achieved with 1 μM phenylephrine. This was followed by the addition of various concentrations (0.01-100 μM) of Acetylcholine (Ach) or sodium nitroprusside (SNP) for the determination of endothelium-dependent relaxation.

Measurement of Biochemical Parameters

Automated Analyzer Roche Cobas c111 was used for the analysis of serum samples of the animals. Commercially available kits were used for the determination of total cholesterol, triglycerides, fasting glucose, total bilirubin, low-density lipoprotein, high-density lipoprotein, direct bilirubin, alkaline phosphatase, uric acid, alanine aminotransferase, creatinine, and urea levels in serum. The serum insulin levels were assessed by means of an enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (CUSABIO, Wuhan, China). All reagents and solutions were prepared on the day of the assay as per manufacturer instructions. The serum samples were set out and allowed to the room temperature. All incubations were carried out at room temperature. All standards, controls, and unknown samples were run in duplicate for the validity of results.

Measurement of Obesity Biomarkers – The obesity biomarkers such as leptin, adiponectin in obesity model were measured *via* ELISA as per manufacturer's recommendation. Blood serum samples were thawed at room temperature for the measurement of leptin and adiponectin[20]. All the assay incubations were carried out at room temperature.

Statistical Analysis

Data are presented as mean ± standard error of the mean. Experiments and treatments were repeated in groups of 5 to 12 rats. Significance between the two groups was evaluated by the Student t-test. Significance between and within multiple groups was evaluated by analysis of variance followed by the Dunnett Multiple Comparison Test. $p>0.05$ was considered significant.

Results

Induction of Obesity

Obesity was induced by feeding animals on high fat diet (60% w/w cholesterol, 20% w/w carbohydrates, and 20% w/w proteins) for two months. Animals received concurrently a single daily oral dose of vehicle or TPPU (2 mg/kg p.o.) for 2 months. Body weights and blood pressure of all animals were registered at 0, 1, and 2 months of the experimental period.

Effect on the Body Weight

As shown in Table I and Figure 1, the body weight of rats fed on a regular diet (normal control) at 0, 1, and 2 months were 168.3±7.5, 249.2±8 and 311.40±9.60 g, respectively. The body weight of vehicle-treated animals fed on a HFD (HFD Control) at 0, 1, and 2 months was 160±6.8, 345.5±10.64, and 446±12.67 g, respectively. HFD diet increased the body weight of animals by 3 fold while animals fed on a regular diet, there was 2 fold increase in their body weight. As seen in Table I, feeding a HFD for two months significantly increased body weight of rats compared to age-matched controls (311.4±9.4 *vs.* 446±12.67 gm, $p<0.001$). The changes in body weight of TPPU (2 mg/kg p.o.) treated animals at 0, 1 and 2 months were 169.20±5.73, 307.80±6.91 and 392.30±11.85 g, respectively. The body weight of animals fed on a HFD was significantly increased ($p<0.001$) at months 1 and 2. As shown in Figure 1, this increase in body weight was attenuated in rats fed on HFD and treated concurrently with TPPU (HFD+TPPU) for two months ($p<0.001$) compared to the vehicle-treated animals fed on a HFD (HFD control).

Table I. Effect of TPPU (2 mg/kg p.o) on total body weight in HFD-induced obesity model in rats.

Month of study	Total body weight (gm)		
	Normal control	HFD control	HFD + TPPU (2 mg/kg p.o)
0 Month	168.3 ± 7.5	160 ± 6.81	169.20 ± 5.73
Month 1	249.2 ± 8.2	345.5 ± 10.64 ***a	307.80 ± 6.91
Month 2	311.40 ± 9.60	446± 12.67 ***b	392.30 ± 11.85***c

Data are presented as mean ± SEM (n=12). Data was analyzed by ANOVA followed by Tukey-Kramer multiple comparison post-test. ***a $p<0.001$compared to HFD control animals (HFD control) at 0 month, **b $p<0.001$ compared to vehicle-treated animals fed on regular diet (Normal control) and TPPU treated animals fed on HFD (HFD+TPPU) after two months of treatment and ***c $p<0.001$ compared to vehicle-treated animals fed on HFD (HFD control) after two months of treatment. TPPU (2 mg/kg p.o.) or vehicle (0.1% Tween 80 in water) was administered concurrently daily for two months.

Effect on Blood Pressure

As shown in Table II and Figure 2, the initial baseline values of blood pressure of either group were not significantly different. There was a significant increase ($p<0.001$) in blood pressure of vehicle-treated animal fed on a HFD (HFD control) compared to the baseline values. The systolic blood pressure of HFD control animals increased from 107.40+1.81 mmHg at 0 month to 130.71+2.67 mmHg ($p<0.001$) after two months (Table II, Figure 2). There was a non-significant increase in the blood pressure from 97.30+2.46 mmHg at 0 month to 111.40+3.68 mmHg at 2 months in animal fed on a regular diet (normal control) (Table II Figure 2). The HFD induced an increase in blood pressure that was inhibited in animals treated concurrently with TPPU (2 mg/kg p.o.) with systolic blood pressure values of 103.20+2.73, 102.80+2.91 and 98.30+1.85 mmHg at 0, 1 and 2 months, respectively. The blood pressure of animals fed on HFD and treated with TPPU was significantly different ($p<0.001$) from vehicle-treated animals fed on HFD (Table II, Figure 2). TPPU treatment reversed the HFD and induced an elevated blood pressure in rats.

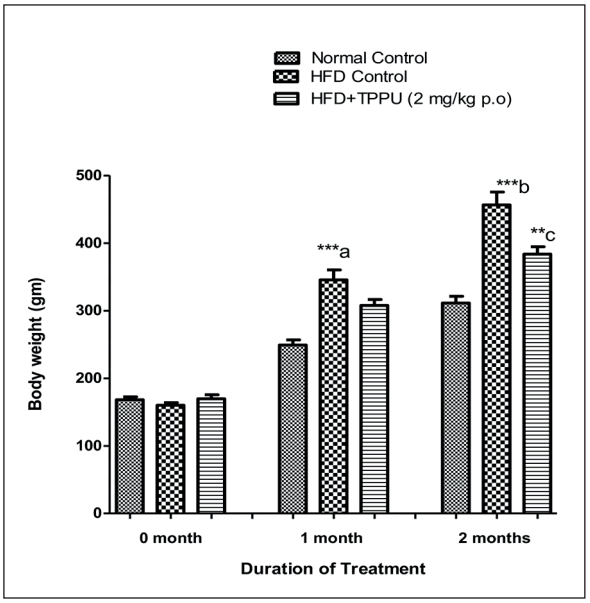

Figure 1. Data are presented as mean ± SEM (n=12) body weight in g. Data was analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison post-test. ***a $p<0.001$compared to HFD control animals (HFD control) at 0 month, **b $p<0.001$ compared to vehicle-treated animals fed on regular diet (Normal control) and TPPU treated animals fed on HFD (HFD+TPPU) after two months of treatment and ***c $p<0.001$ compared to vehicle-treated animals fed on HFD (HFD control) after two months of treatment. TPPU (2 mg/kg p.o) or vehicle (0.1% tween 80 in water) was administered concurrently daily for two months.

Effect on Vascular Reactivity in Isolated Thoracic Aorta

Vascular reactivity was assessed in the isolated rat thoracic aorta from control animals fed on a regular diet (Normal control) or a HFD treated with vehicle (HFD control) or TPPU (2 mg/kg p.o.). The relaxant response of the isolated vessel, pre-constricted with phenylephrine (10^{-4} M) to cumulative addition of sodium nitroprusside (SNP) or Acetylcholine (Ach) [10^{-9} to 10^{-5} M] was measured (Figure 3). As shown in Figure 3 A and B, SNP and Ach caused concentration-dependent relaxation of the vessels isolated from the control animals fed on a regular diet with maximum relaxations of 92.6±3.4 and 91.3±4.5%, respectively. Aortic rings isolated from vehicle-treated animals and fed on a HFD had a blunted response to SNP and Ach with maximum relaxations of 62.8±1.8 and 60.2±3.2%, respectively (Figure 3 A and B). Aortic rings prepared from animals

Table II. Effect of TPPU on systolic blood pressure in high fat diet induced obesity model in rats.

Month of study	Blood pressure (mmHg)		
	Normal control	HFD control	HDF + TPPU (2 mg/kg p.o)
0 Month	97.30 ± 4.46	107.40 ± 1.81	103.20 ± 2.73
Month 1	104.50 ± 2.82	117.5 ± 1.64**[a]	102.80 ± 2.91
Month 2	111.40 ± 3.68	130.71 ± 2.67***[b]	98.30 ± 1.85**[c]

Data are presented as mean ± SEM (n=12). Data was analyzed by ANOVA followed by Tukey-Kramer multiple comparison post-test. **[a] $p<0.01$compared to HFD control animal at 0 month, ***[b] $p<0.001$ compared to vehicle-treated animals fed on regular diet (Normal control) and TPPU treated animals fed on a HFD (HFD+TPPU) after two months of treatment and ***[c] $p<0.001$ compared to vehicle-treated animals fed on a HFD (HFD control) after two months of treatment.

fed on a HFD and treated with TPPU (2 mg/kg p.o.) for two months showed a significant ($p<0.001$) improvement in the relaxant response to SNP or Ach compared to the response in vessels isolated from HFD control animals (Figure 3 A and B). The relaxant response of the vessels isolated from TPPU treated animals was reversed to normal with maximum relaxations of 91.7±4 and 90.2±3.5 to SNP and Ach, respectively (Figure 3 A and B). The relaxant effect of SNP or Ach in isolated thoracic aorta from animals fed on a HFD and TPPU treated was significantly different ($p<0.001$) compared to vehicle-treated animals fed on a HFD.

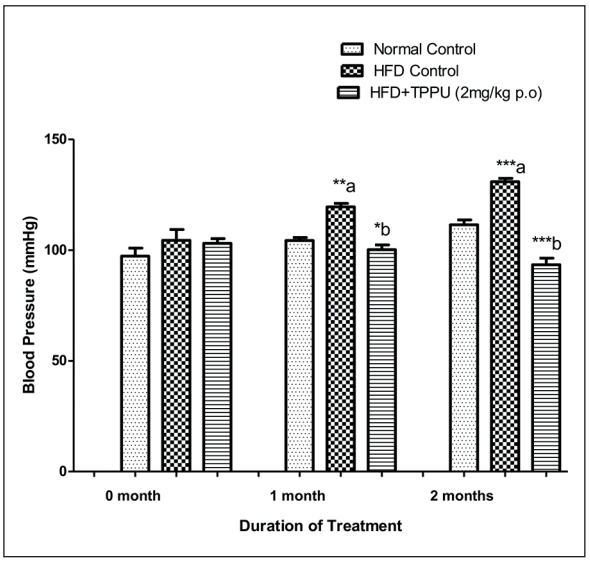

Figure 2. Data are presented as mean ± SEM (n=12). Data was analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. **a $p<0.01$ compared to HFD control animals (HFD control) at 0 month, ***a $p<0.001$ compared to HFD control animals at 0 month, *b $p<0.05$ compared HFD control animals after 1 and ***b $p<0.001$ compared to HFD control at 2 months of treatments. TPPU (2 mg/kg p.o) or vehicle (0.1% Tween 80 in water) was administered concurrently, daily for two months.

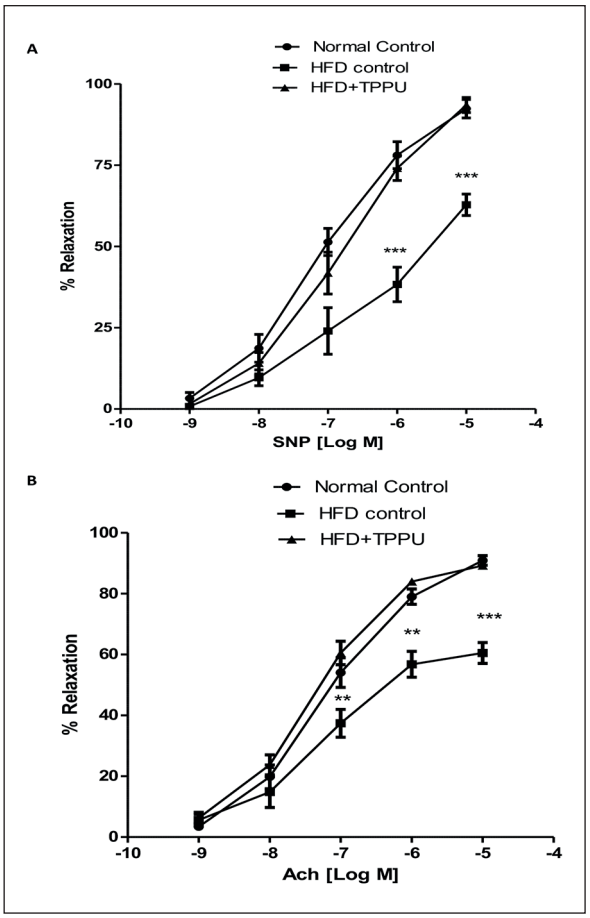

Figure 3. Vasorelaxant effect of sodium nitroprusside (SNP) (A) and Acetylcholine (Ach) (B), in rat's thoracic aorta. Data are presented as mean ± SEM (n=8). Data was analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. **$p<0.01$, *** $p<0.001$ compared to HFD control animals. TPPU (2 mg/kg p.o) or vehicle (0.1% Tween 80 in water) was administered daily for two months.

Effects on Biochemical Parameters

As shown in Figures 4-5, vehicle-treated animal fed on a high fat diet (HFD control) for two months exhibited significant changes in various biochemical parameters measured in the plasma compared to those fed on a regular diet (Normal control). HFD resulted a significant (p<0.001) increase in plasma cholesterol from 1.34±0.05 at 0 month to 1.85±0.1 and 1.97±0.08 mmol/L at 1 and 2 months, respectively. As expected the plasma triglycerides (TGs) were also significantly (p<0.001) increased from 0.80±0.08 at month 0 to 1.30±0.12 and 1.38±0.13 mmol/L at month 1 and 2 of HFD, respectively (Figure 4 B). Similarly, plasma levels of low density lipids (LDL) were significantly elevated from 0.15±0.03 at 0 month to 0.31±0.04 and 0.30 ± 0.06 mmol/L at month 1 and 2, respectively (Figure 5A). As shown in Figure 5 B, HFD diet resulted a significant decrease in plasma levels of high density lipids (HDL). The observed HDL levels of vehicle-treated animals and fed on a HFD at month 0, 1, and 2 were 1.3±0.03, 1.00±0.04, and 1.03 ± 0.05 mmol/L, respectively. There was a tremendous increase in plasma creatinine levels of animals fed on a HFD compared to those fed on regular diet. HFD produced a highly significant increase (p<0.001) in the plasma creatinine levels of animals from 37.72±0.9 mmol/L at month 0 to 50.4±1.1 and 58.7±1.4 umol/L at month 1 and 2, respectively (Figure 6). No significant change was observed in the blood glucose levels of animals fed on a HFD or a regular diet (Figure 7). There was a highly significant

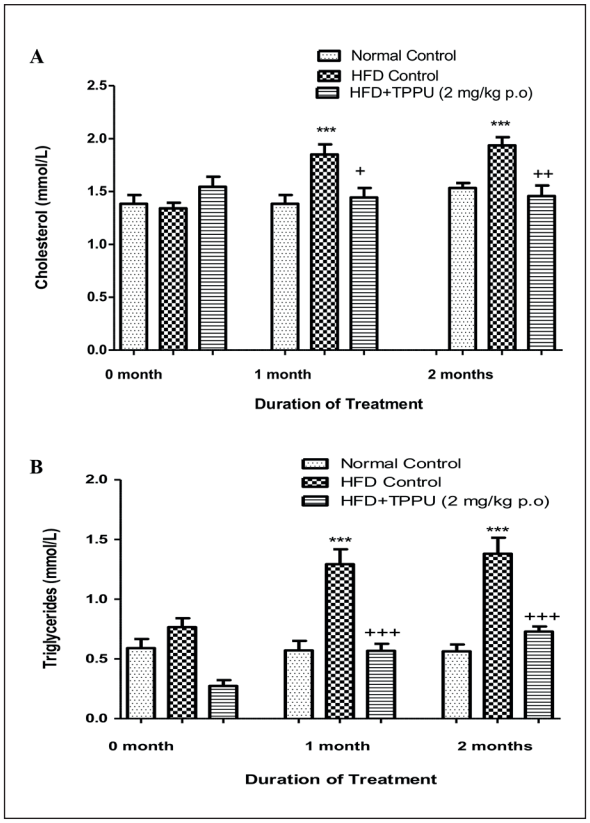

Figure 4. Data are presented as mean ± SEM (n=12) cholesterols **(A)** and Triglycerides **(B)**. Data was analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. ***p<0.001 compared to HFD control animal at 0 month, +p<0.05, ++p<0.01, +++p<0.001 compared to HFD control animals at 1 month and 2 months. TPPU (2 mg/kg p.o) or vehicle (0.1% tween 80 in water) was administered concurrently daily for two months.

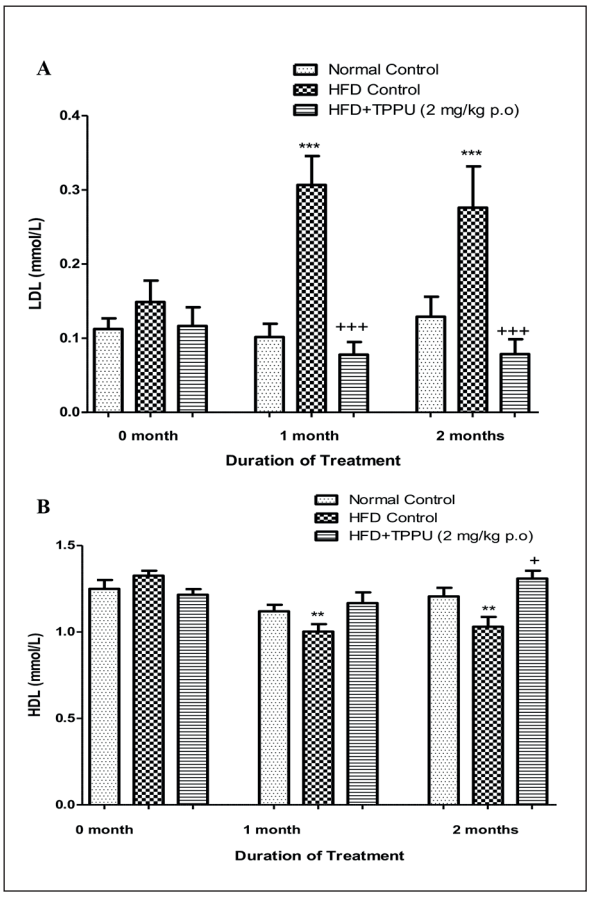

Figure 5. Data are presented as mean ± SEM (n=12) low density lipids (LDL) (A) and high denisity lipids (HDL) (B). Data was analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. **p<0.01, ***p<0.001 compared to HFD control animal at 0 month, + p<0.05, +++ p<0.001 compared to HFD control animals at 1 month and 2 months. TPPU (2 mg/kg p.o) or vehicle (0.1% tween 80 in water) was administered daily for two months.

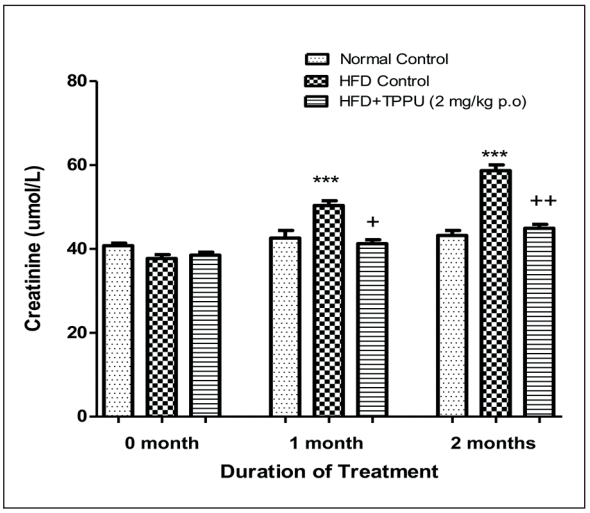

Figure 6. Data are presented as mean ± SEM (n=12). Data was analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. ***p<0.001 compared to HFD control animal at 0 month, +p< 0.05, +++p<0.001 compared to HFD control animals at 1 month and 2 months. TPPU (2 mg/kg p.o) or vehicle (0.1% tween 80 in water) was administered daily for two months.

(p<0.001) increase in the alanine transaminase (ALT) and alkaline phosphatase (ALP) levels of animals fed on a HFD compared to those fed on a regular diet (Figure 8 A and B). ALT values in animals fed on a HFD increased from 63.6±2.7 at month 0 to 90.5±6 and 108.4±8.3 U/L at month 1 and 2, respectively (Figure 8A). As shown in Figure 8B, similar increase in the ALP levels occurred in animals fed on a HFD. ALP values in animals fed on a HFD increased from 116.5±3.76 at month 0 to 147.8±6.6 and 171.4±10.1 U/L at month 1 and 2, respectively (Figure 8 B). There was no appreciable change in any of the above-mentioned biochemical parameters in animals fed on a regular diet for two months.

As shown in Figure 4-8, Animals fed on a HFD diet and treated concurrently with TPPU (2 mg/kg p.o.) daily for two months caused a significant (p<0.01, p<0.001) reduction in all biochemical parameters except glucose levels. There was a marked decrease in plas-

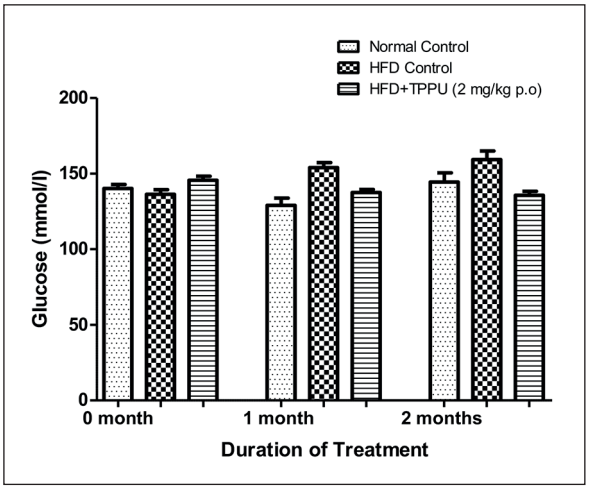

Figure 7. Data are presented as mean ± SEM (n=12). Data was analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. TPPU (2 mg/kg p.o) or vehicle (0.1% Tween 80 in water) was administered daily for two months.

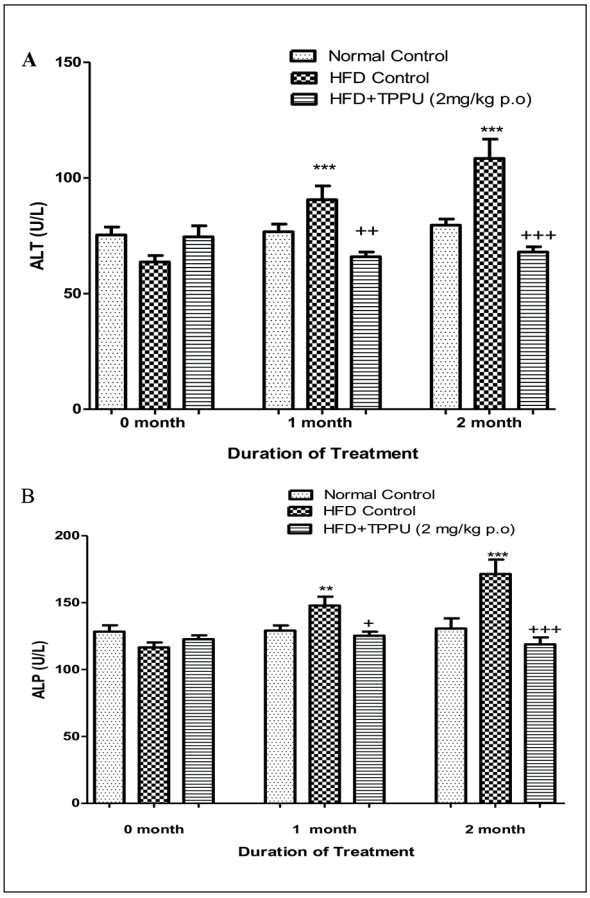

Figure 8. Data are presented as mean ± SEM (n=12), alkaline transaminase (ALT) **(A)** and alkaline phosphatase (ALP) **(B)**. Data was analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. **p<0.01, ***p<0.001 compared to HFD control animal at 0 month, +p<0.05, ++p<0.01, +++p<0.001 compared to HFD control animals at 1 month and 2 months. TPPU (2 mg/kg p.o) or vehicle (0.1% Tween 80 in water) was administered daily for two months.

ma cholesterol and triglycerides (TGs) from 1.97±0.08 to 1.46±0.1 mmol/L and 1.38±0.13 to 0.73±0.04 mmol/L at 2 months of TPPU treatment, respectively (p<0.001) compared to HFD Control vehicle-treated animals, Figure 4 A and B. TPPU treated animals also showed a highly significant reduction in the plasma LDL levels (0.28±0.06 mmol/L, HFD control *vs*. 0.08 ± 0.02 mmol/L, TPPU treated animals, p<0.001) (Figure 5 A). As shown in Figure 5 B, a significant increase in HDL levels (p<0.05) was observed in animals fed on a HFD and treated with TPPU compared to vehicle-treated animals fed on a HFD. A significant (p<0.01) improvement was seen in the creatinine levels of TPPU treated animals fed on a HFD. Plasma creatinine values of HFD control and TPPU treated animals were 58.69±1.4 and 45±0.92 umol/L respectively after 2 months of treatment (Figure 6). As shown in Figure 7, there was no significant difference in the blood glucose level of animals in either group. Both ALT and ALP levels were significantly (p<0.001) decreased in TPPU treated animals fed on a HFD diet compared to their respective HFD control animals (Figure 8A and B).

Effect on Markers of Obesity

Baseline values (0 month) of leptin, adiponectin and insulin levels were not significantly different in all groups of animals (Figures 9-11). Fasting serum adiponectin levels were significantly decreased (p<0.001) at 1 and 2 months' treatment of HFD compared to its respective normal control at 0 month. As shown in Figure 9, the fasting serum adiponectin levels of vehicle-treated animals and fed on a HFD were reduced from 29.5±3.1 ng/mL at 0 month to 22.5±2.0 and 20.4±2.5 ng/mL at 1 and 2 months, respectively. Animal fed on a HFD and treated concurrently with TPPU (2 mg/kg p.o.) for two months showed a significant increase (p<0.001) in adiponectin levels from 27.5±2.5 ng/mL at 0 month to 30.2±1.8 and 34.1±1.7 at 1 and 2 months, respectively (Figure 9).

As shown in Figure 10, a significant increase (p<0.001) in the leptins levels of vehicle-treated animals fed on HFD with mean leptin value of 0.74±0.21, 12.41±1.4 and 16.8±1.82 ng/mL at 0, 1 and 2 months, respectively. Animals fed on HFD and treated concurrently with TPPU (HFD+ TPPU 2 mg/kg p.o.) had marked reduction in leptin levels after 1 and 2 months of treatment (p<0.001) (Figure 10). Leptin levels did not vary in vehicle-treated animals fed on a regular diet (Normal control) (Figure 10). The mean leptin levels of animals in normal control group were 1.17±0.2, 1.60±0.4 and 1.61±0.23 at 0, 1 and 2 months of treatment, respectively (Figure 10). As shown in Figure 10, Leptin remained unchanged in vehicle-treated animals fed on a regular diet for two months.

As shown in Figure 11, the plasma insulin levels at baseline (0 month) were not significantly different in animals fed on a regular diet (Normal control) or HDF + vehicle-treated animal (HFD control) or animals fed on HFD diet and treated concurrently with TPPU at the dose of 2 mg/kg p.o. (HFD +TPPU) for two months. The insulin levels of animals fed on HDF were significantly increased (p<0.001) from 2.1±0.4 ng/mL at 0 month to 4.43±0.46 and 4.60±0.6, at 1 and 2 months respectively. The insulin levels of animals of HFD+TPPU group were tremendously decreased after two months of treatment (Figure 11). As shown in Figure 11, the insulin levels of animals of HFD+TPPU were significantly (p<0.01; p<0.001) different at 1 and 2 months compared to vehicle-treated animals fed on a HFD. Concurrent treatment of animals with

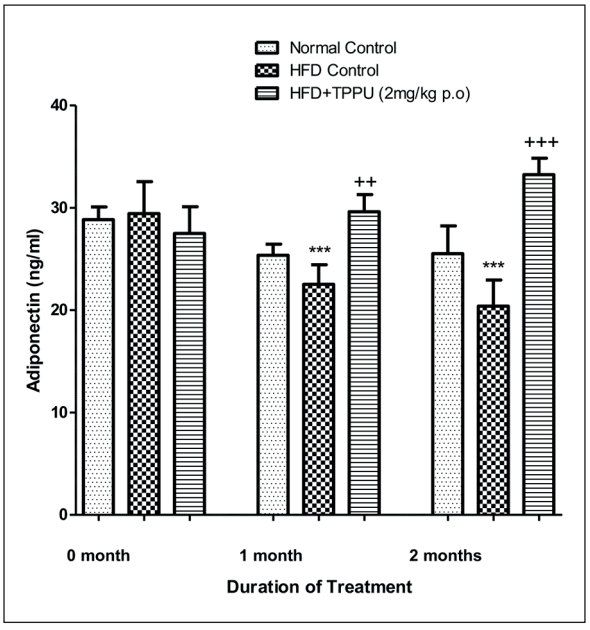

Figure 9. Data are presented as mean ± SEM (n=12) and analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. ***p<0.001 compared to HFD control animal at 0 month, ++p<0.01, +++p<0.001 compared to HFD control animals at 1 month and 2 months. TPPU (2 mg/kg p.o) or vehicle (0.1% Tween 80 in water) was administered daily for two months.

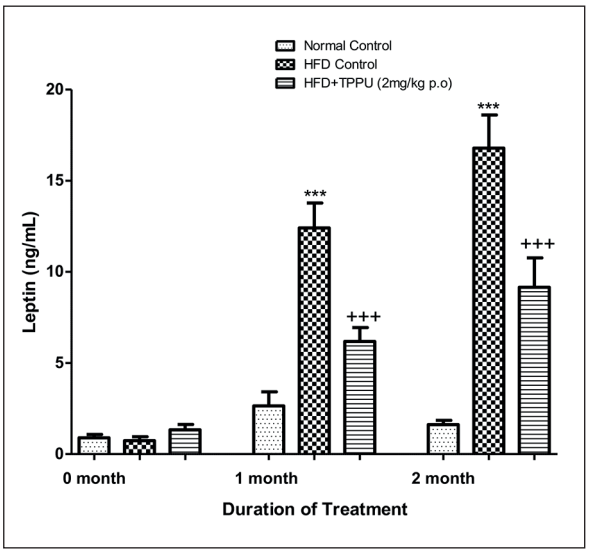

Figure 10. Data are presented as mean ± SEM (n=12) and analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. **p<0.01, ***p<0.001 compared to HFD control animal at 0 month, +++p<0.001 compared to HFD control animals at 1 month and 2 months. TPPU (2 mg/kg p.o) or vehicle (0.1% Tween 80 in water) was administered daily for two months.

TPPU reduced serum insulin levels of animals from 4.43±0.5 to 2.81 ± 0.31 ng/mL at 1 month and from 4.57±0.43 to 2.7±0.23 ng/mL after 2 months of TPPU treatment (Figure 11).

Discussion

In the present study rats fed on a high fat diet (HFD: cholesterol 40%, carbohydrates 40% and proteins 20%) became obese after 2 months of feeding on a HFD. Body weight of animals fed on a HFD was significantly increased compared to age-matched control animals fed on a regular diet. This gain in body weight was attenuated in rats fed on a HFD and treated concurrently with TPPU, a soluble epoxide hydrolase inhibitor (sEHI), for two months (p<0.01). These findings are in parallel with earlier studies showing that sEHIs promoted weight loss by reducing appetite and increasing metabolic rate in high-fat diet-treated mice[21]. Systolic blood pressure was significantly increased in the rats fed on a HFD compared to control rats fed on a regular diet. Aligned with earlier studies, in the current investigation, HFD caused vascular dysfunction and hypertension along with other metabolic disorders in rats[6,22]. This may have occurred *via* decreased endogenous levels of EETs in obese animals[8]. The HFD-induced an increase in blood pressure in rats that was attenuated (p<0.01) by concurrent treatment with TPPU, further strengthening the speculation that increasing EETs levels by sEHIs is reasonable for its anti-hypertensive effects in obese rats[13,23]. Increasing the levels of EETs by the administration of AUDA or TPPU, sEHIs, can cause dilation and decreased resistance of renal blood vessels and contribute to the correction of abnormal renal hemodynamics in HFD fed obese rats[12,15,24]. Moreover, some studies[25,26] have analyzed alterations in CYPs 450 metabolites contributing to renal damage in obesity and diabetes leading to an increase in blood pressure in animals fed on a HFD. In the present study HFD caused a significant increase (p<0.001) in plasma creatinine levels in animals compared to those fed on a regular diet. The increase in plasma creatinine levels was inhibited in animals fed on a HFD and treated concurrently with TPPU for two months. Other study[27] has shown that genetic disruption of sEH in STZ-induced diabetic mice showed significant decreased levels of creatinine.

One of the hall marks of obesity includes endothelial dysfunction which is manifested as impaired vasorelaxant response to acetylcholine

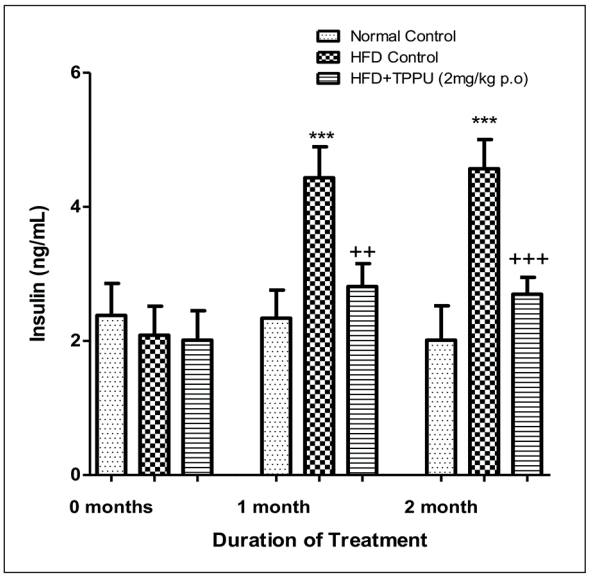

Figure 11. Data are presented as mean ± SEM (n=12) AND analyzed by on way ANOVA followed by Tukey-Kramer multiple comparison post-test. ***p<0.001 compared to HFD control animal at 0 month, ++p<0.01, +++p<0.001 compared to HFD control animals at 1 month and 2 months. TPPU (2 mg/kg p.o) or vehicle (0.1% Tween 80 in water) was administered daily for two months.

or sodium nitroprusside (SNP). It was previously reported that the vasorelaxation in response to acetylcholine was severely impaired in obese animals[23]. Treatment of animals with sEHIs prevented endothelial dysfunction in obese animals[23]. In the present investigation, the relaxant response of vessel to acetylcholine and SNP was significantly impaired in vehicle-treated animals fed on a HFD for two months compared to aged matched control animals fed on a regular diet. Consistent with earlier reports[23,28], in our study, the animals fed on a HFD and treated concurrently with TPPU for two months restored maximal relaxation to acetylcholine and SNP similitude to that recorded in control animals fed on a regular diet. It is a well established fact that EETs function as endothelium derived hyperpolarizing factor (EDHF) causing vaorelaxation[19,29,30]. EETS levels are decreased in obese animals and are positively correlated with impaired vasorelaxant response to acetylcholine in animals fed on HFD[31]. Clinically, obesity is significantly associated with low plasma EET levels, suppressed CYP epoxygenase activity, and metabolic activity of sEH is enhanced in obese individuals[8,32]. These findings demonstrate that inhibition of sEH can provide therapeutic benefit by improving endothelial function and decreased blood pressure in HFD fed obese animals.

High fat diet-induced obesity is frequently associated with systemic inflammation. In the current study, the circulating obesity markers were markedly increased in obese untreated animals. Plasma adiponectin levels were significantly lower (p<0.001) in vehicle-treated rats fed on a HFD for two months compared to control animals fed on a regular diet (p<0.001). Consistent with earlier reports, in the current study, the plasma adiponectin levels were increased in rats fed on a HFD and treated concurrently with TPPU or sEHIs[3,33]. Leptin resistance is also common in obesity[34]. Most obese individuals have higher leptin levels compared to normal non-obese subjects and are resistant to the effects of leptin[35,36]. In our study, the leptin levels were drastically increased in animals fed on a HFD compared to those fed on a regular diet. The increase in leptin levels was significantly decreased in animals fed on a HFD and treated concurrently with TPPU for two months. Previous study[25] has also shown the increase in leptin levels in animals fed on HFD. Moreover, this study also revealed that elevated leptin levels were positively linked to increased blood pressure in HFD induced obesity model in rodents. These findings demonstrate that the observed decrease in leptin levels in our study may have attenuated the HFD-induced an elevated blood pressure in TPPU treated animals in addition to vasodilator effects of increased level of EETS due to sEH inhibition.

Obesity exhibits a higher risk of type 2 diabetes wherein a number of tissues are insulin resistant[37,38]. As expected, in the current study, rats fed on a HFD showed increased levels of plasma insulin compared to the control animals fed on a regular diet. This increase in insulin levels was significantly suppressed by TPPU treatment in rats fed on a HFD. EETs and their metabolizing enzyme, sEH, play an important role in the development and progression of insulin resistance. Decreased levels of EETs leads to attenuated insulin sensitivity based on observations that cytochrome P450 (CYP2C) expression is decreased and sEH expression is increased in obese Zucker rats, a commonly employed animal model of obesity and insulin resistance[39,40]. In the present study, though there was a tremendous increase in insulin resistance, the blood glucose levels were not affected by TPPU treatment. The blood glucose levels were slightly increased in rats fed a HFD compared to those on a regular diet. There was a slight decrease in blood glucose levels in rats fed on a HFD and treated with TPPU. It is possible that HFD induced obesity and the resulted insulin resistance needs to be maintained for a longer span to see its impact of on the glucose homeostasis.

Dyslipidemia is also related to obesity with increased TGs, LDLs, and reduced HDL[11]. In the current study, there was a drastic increase in plasma cholesterol, TGs, LDLs levels in rats fed on a HFD. HFD cuuased derangement of the levels of serum liver enzymes, ALT, and ALP. Concurrent administration of TPPU to animals fed on a HFD ameliorated dyslipidemia and reversed HFD induced ALT and ALP abnormalities in rats. Enhancing EETS levels through cytochrome CYP2J2 overexpression or Inhibition of sEH was found to lower total cholesterol and TGs[41]. A marked decrease in plasma cholesterol was observed in sEH knockout male mice compared with wild-type male mice[40]. The observed inhibitory effects of TPPU against HFD-induced dyslipidemia are beneficial to suppress atherosclerotic process[41,42]. Furthermore, the observed inhibitory effect of TPPU in the current investigation on leptin resistance in obese animals may be attributed to improved cardiovascular and metabolic functions in these animals. Leptin

prevents the desire for further food intake *via* binding to specific receptors on appetite-modulating neurons[43]. Obese people have elevated level of leptin, indicating leptin resistance. In the current investigation, TPPU markedly decreased leptin resistances which may explain its effect on decreasing body weight of obese animals. These findings indicate that TPPU could be potential new drug to manage obesity induced cardiovascular and metabolic complications.

To our knowledge this the first study that has explored the role of soluble epoxide hydrolase inhibitor (TPPU) in the animal model of high fat diet induced obesity and related cardiovascular and metabolic disorders. The findings from our study revealed promising preventive and/or therapeutic potential of TPPU. Further studies are warranted to investigate the dose dependent effect of soluble epoxide hydrolase inhibitors on high fat diet induced cardiovascular and metabolic disorders and production of epoxyeicosatrienoic acids (EETs) with special emphasis on their correlation with behavior of some relevant hormones (leptin, and visfatin) and histopathological changes at hepatic and cardiac cells.

Conclusions

In the present study, we have demonstrated that feeding animals a high fat diet (HFD) for two months induced obesity that subsequently produced a variety of cardiovascular and metabolic disorders. HFD caused a substantial increase in the body weight and blood pressure of the animals compared to animals fed on a regular diet. HFD-induced obesity impaired the endothelium function with attenuated vasodilator response to acetylcholine and sodium nitroprusside. Concurrent treatment of animals daily with TPPU (2 mg/kg p.o) and fed on a HFD for two months attenuated the obesity induced increase in body weight and blood pressure in rats. Vascular reactivity was restored to normal in animals fed a HFD and receiving oral TPPU for two months. HFD diet caused tremendous derangements in the biochemical and metabolic profile of untreated animals. Plasma levels of creatinine, cholesterol, TGs, LDL, ALT, ALP, and glucose were increased while HDL levels decreased in vehicle-treated animals fed on a HFD. These HFD induced an increase in the metabolic parameters was attenuated in animals treated concurrently with TPPU and fed on a HFD. Concurrent administration of TPPU attenuated the HFD-induced insulin and leptin resistance and increased cardio-protective adiponectin levels in obese animals. This study revealed the potential therapeutic benefits of TPPU against obesity induced cardiovascular and metabolic complications. TPPU is a sEH inhibitor that works by decreasing metabolism of Epoxyeicosatrienoic acids (EETs) and increasing their plasma levels. EETS are CYP epoxygenase metabolites of arachichidonic acid, which participate in regulating blood pressure, inflammatory cascades, and glucose homeostasis. The beneficial effects of TPPU on blood pressure, vascular endothelium function, insulin resistance, dyslipidemia and other metabolic disorders observed in our study could be related to its action on enhancing and stabilizing EETs function in obese animals. The discovery of selective inhibitors of sEH and sEH knockout animal models has increased our understanding of the physiological role of EETs/sEH in obesity and related complications. Which in turn provides exciting avenue to develop new drugs for the treatment of obesity-induced complication in humans. The findings from our study warrant further studies to define the precise signaling mechanisms affected by EETs in obesity.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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