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ORIGINAL ARTICLE

Protective Effect of Adropin against High Fat Diet-induced Obese Diabetic Wistar Rats via Nuclear Factor Erythroid 2-related Factor 2 Pathway

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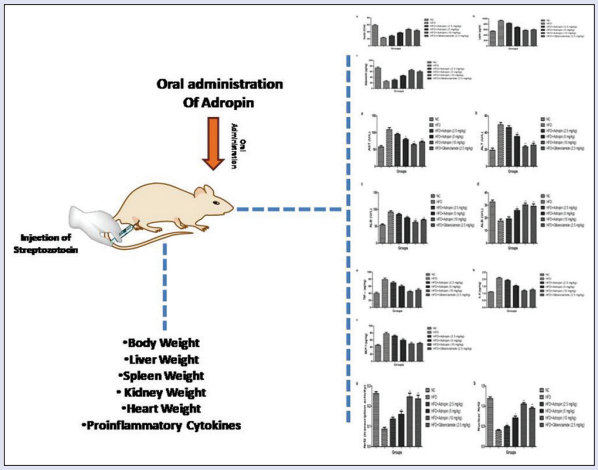
ABSTRACT

Introduction: Liver steatosis (fatty liver) is frequently found during the conditions such as diabetes and obesity. The current experimental study was executed the effect of adropin against high fat diet (HFD)-induced obese diabetic Wistar rats via nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. **Materials and Methods:** Wistar rats were randomly divided into six groups and divided as follows: normal, HFD control, HFD + adropin (2.5, 5 and 10 mg/kg), and HFD + Glibenclamide (2.5 mg/kg), respectively. Lipid and carbohydrate metabolism, hepatic parameters, antioxidant, and proinflammatory cytokines were estimated at the end of the experimental study. Nrf2 transcription and nuclear level were also estimated. *In vitro* adropin diminished the accumulation of lipid droplets in dose-dependent manner and no effect was observed on the lipolysis. Type II diabetic rats fed with HFD exhibited a marked reduction in hepatic extraction faction and hepatic steatosis after the adropin and glibenclamide treatment. **Results:** Adropin significantly ($P < 0.001$) altered the hepatic parameter such as alanine transaminase, aspartate transaminase, alkaline phosphatase; antioxidant parameters such as thiobarbituric acid reactive substances, 8-OhdG, superoxide dismutase, glutathione (GSH) peroxidase, catalase, GSH, GSH reductase, GSH S-transferase; proinflammatory cytokines, namely tumor necrosis factor-α, interleukin-6, and monocyte chemoattractant protein-1, respectively. In addition, adropin significantly reduced the nuclear Nrf2 activity at dose-dependent manner. **Conclusion:** Adropin improved insulin sensitivity, reduced lipogenesis in the adipocytes and also decrease the inflammation through downregulated cytokines (Nrf2 pathway).

Key words: Adropin, antioxidant, nuclear factor erythroid 2-related factor 2 pathway, obesity, proinflammatory cytokines

SUMMARY

- Adropin increased insulin sensitivity, decreased lipogenesis in the adipocytes and also minimized inflammation through down-regulated cytokines.



Abbreviations used: FBG: Fasting blood glucose; HFD: High fat diet; ITT: Insulin tolerance test; ALT: Alanine transaminase; Nrf2: Nuclear factor erythroid 2-related factor 2; ALP: Alkaline phosphatase; AST: Aspartate transaminase; TBARS: Thiobarbituric acid reactive substances; GSH: Glutathione; GST: Glutathione S-transferase; SOD: Superoxide dismutase; 8-OhdG: 8-Oxo-2′-deoxyguanosine; CAT: Catalase; GR: Glutathione reductase; GPx: Glutathione peroxidase; TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; VLDL: Very low-density lipoprotein; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; MCP-1: Monocyte chemoattractant protein-1; LPO: Lipid peroxidation; NASH: Non-alcoholic steatohepatitis; WAT: White adipose tissue; STZ: Streptozotocin; OGTT: Oral glucose tolerance test; ELISA: Enzyme-linked immunosorbent assay; PCR: Polymerase chain reaction; ANOVA: One-way analysis of variance.

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INTRODUCTION

It is well documented that lipid peroxidation (LPO) has been play an important role in the non-alcoholic steatohepatitis (NASH).[1] The previous investigation suggest that the LPO is the crucial mechanism that encourages the expansion of steatosis to steatohepatitis, which further boost the inflammatory reaction.[1,2] Based on the previous study, LPO can be showed in steatosis, but the expansion of steatosis to steatohepatitis in the human is not regular feature, the expansion of steatosis to steatohepatitis need various factor.[3] Few studies suggest

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that the high-fat diet (HFD), oxidative stress, inflammation, and insulin resistance are the significant factors, played a crucial role in the pathogenesis of human NASH.[3-5]

Previous research suggests that fatty liver (liver steatosis) is commonly observed in conditions such as diabetes and obesity.[6,7] Although steatosis leads to steatohepatitis and liver cirrhosis,[8] obesity is linked with diabetes mellitus, dyslipidemia, cardiovascular disease, and hypertension. Diabetes mellitus (type II) is an increasingly common disorder of lipid and carbohydrate metabolism. The accumulation of fats on adipose tissue earlier than hepatic tissue is the main indication of obesity and its induced inflammation in the body. The human body contains numerous fat deposits, which can be further grouped into brown and white fats. White adipose tissue (WAT) is multifunctional organs which store the nutrients in the form of fat droplets and also release the cytokines that affect the body metabolic state.[9,10] It also play a crucial role in the endocrine system in the body. Excess energy intake and fat deposition induce the hyperplasia and adipocyte hypertrophy, which may lead to the progression of HFD-induced obesity.[11,12] During the obesity, hypertrophic adipocytes secrete the pro-inflammatory cytokines and chemokines to activate and fascinate the inflammation cells into the WAT. These processes involved in the systemic insulin resistance and finally expand the state of chronic low-grade adipose tissue inflammation.[12,13]

There has been a tragic increase in diabetes across the world, paralleling the overweight and obesity epidemic.[14] It is well known that the patient of diabetes mellitus (type II) increases day by day; lipid and carbohydrate metabolism are the common factors for this disease.[15,16] The Asian people having the high risk of cardiovascular disease and diabetes associated with obesity due to the predisposition to abdominal obesity.[17,18] These diseases lead to the impairment of glucose tolerance and metabolic syndrome.[19,20] Moreover, in the World population, approximately 197 million people have impairment in glucose tolerance due to expansion of obesity and its related metabolic syndrome.[14,21]

It is well documented that adropin is the energy homeostasis-related gene and has been proposed to be released protein.[22,23] Previous studies suggest that the dietary nutrients can alter the expression of gene and also circulating the level of adropin.[22,23] Studies also suggest that the adropin can enhance the dyslipidemia and glucose homestasis in the obesity mice.[24,25] Moreover, adropin has been reduced the PDK4 expression and boost glucose utilization.[24] It is well documented that non-alcoholic fatty liver disease and reactive oxygen species play a significant role in the expansion of NASH.[24] In the current experimental study, we hypothesized that adropin exhibited the anti-obesity effect against HFD-induced obese diabetic Wistar rats via nuclear factor erythroid 2-related factor 2 (Nrf2) pathway.

EXPERIMENTAL DATA

Chemicals

Streptozotocin (STZ) was purchased from the Sigma Aldrich, U.S.A. Malondialdehyde, superoxide dismutase (SOD), catalase (CAT), GSH, and urinary 8-hydroxydeoxyguanosine (8-OHdG) were estimated using the enzyme-linked immunosorbent assay (ELISA) (OXIS health Products, Portland, OR, USA). Nrf2-binding activity was estimated using the TransAM Nrf2 kit (Active Motif). Proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and IL-1 β (R and D Systems Europe Ltd., UK) were estimated using the standard kits.

In vivo experiment

Animals

For the current experimental study, Wistar rats (175–200 g, male) 7-week-old were used from the current experimental study. The rats were kept in the standard laboratory in the polyethylene cage in the standard experimental condition (22 ± 3°C temperature, relative humidity 60 ± 5%, and 12/12 h dark/light cycle). The rats received the standard diet pellet diet and water *ad libitum*.

Tolerance tests

For oral glucose tolerance test, briefly, the all group rats were fasted overnight for 16 h and the blood glucose level were estimated at regular time interval (0–120 min) after the oral administration of glucose (2.0 mg/kg) through gastric tube. The blood glucose level was estimated using the glucose estimated kits (Johnson and Johnson, U.S.A).

Insulin tolerance test was performed on the fasted (3 h) rats. Briefly, for the estimation of blood glucose level, the blood samples were collected from the tail vein before at regular time intervals after the intraperitoneal administration of insulin (0.75 U/kg, body weight). The blood glucose level was estimated using the Glucose estimated kits (Johnson and Johnson, U.S.A).

Preparation of streptozotocin

For induction of diabetes mellitus, the solution of STZ (55 mg/kg) were dissolved in the freshly prepared citrate buffer (pH = 4.5) and stored in the cold condition until the use.[26,27]

Induction of diabetes

For the current study, the rats were fed with the HFD (consisting of cholesterol (25 g), lard (100 g), sodium deoxycholate (10 g) sucrose (5 g) with ordinary fodder). After 4 weeks, the rats were administered with intraperitoneal injection of STZ (40 mg/kg) and used for the induction of diabetes. After 7 days, the blood sample was collected via puncturing the retro-orbital plexus of all group rats for the estimation of the blood glucose level. The rats had fasting blood glucose level >16.7 mmol/L were considered as diabetic.[26,28,29]

Experimental model

For the diabetic model, the rats were randomly divided into the following groups and each group contains 12 animals. The groups were the following:

- Group I: administered saline only (normal rats)
- Group II: administered saline only (diabetes control rats)
- Group III: administered Adropin (2.5 mg/kg) (treated group rats with low dose)
- Group IV: administered Adropin (5 mg/kg) treated group rats with intermediate dose)
- Group V: administered Adropin (10 mg/kg) (treated group rats with high dose)
- Group VI: administered glibenclamide (2.5 mg/kg) (standard drug), respectively.

Moreover all the group rats, continue received the above-discussed treatment for 8 weeks. Blood glucose level of all group rats were estimated at regular interval. After the 8 weeks, all the group rats were placed in the individual metabolic cages and collected the urine for 24 h. Water intake, food, and body weight were recorded at regular intervals.[29]

After the 8 weeks, rats were fasted overnight (12 h) and blood samples were collected for estimation of the biochemical parameters and finally, the rats were sacrificed via using the excess of anesthesia and renal and hepatic tissues were also collected.

Serum parameters

The glucose level, triglycerides (TGs), and nonesterified fatty acids were estimated via using the enzymatic colorimetric methods. Adiponectin and serum insulin levels were estimated using the rat insulin and mouse/rat adiponectin ELISA kits, respectively.

Hepatic parameters

Hepatic parameters such as albumin (ALB), alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) were estimated via using the standard ELISA kits as per mention in the manufacture instruction.

Antioxidant parameters

Antioxidant parameters such as CAT, SOD, glutathione (GSH), GSH S-transferase (GST), GSH peroxidase (GPx), GSH reductase (GR), thiobarbituric acid reactive substances (TBARS), and 8-Oxo-2'-deoxyguanosine (8-OhdG) were estimated via using the manufacture instruction of the standard ELISA kits.

Lipid parameters

Lipid parameters such were estimated via using the manufacture instruction of standard ELISA kits.

RNA preparation and reverse transcription-polymerase chain reaction quantification

Guanidine isothiocyanate-based reagents isogen was used for the total RNA from the muscle tissue via using the manufacture's instruction (Nippon Gene, Tokyo, Japan). The isolated RNA was treated with Rnas free Dnase at 37°C for 10 min prior use of RNeasy Mini Kitrs (Qiagen) and estimation of the absorbance at 260 and 280 nm wavelengths. Finally, the agarose gel electrophoresis was performed for isolated RNA for the qualitative analysis. Total RNA (4 mg) was used to synthesize the cDNA. The isolated total RNA was denatured in the presence of oligo, dNTP (10 mmol/L) and primers at 65°C for 5 min and incubated in 20-μL volume with Tris HCl (50 mmol/L) containing DTT (0.1 mol/L) superScript III reverse transcriptase inhibitor (50U) at 25°C for 5 min 60 min at 50°C and 15 min for 70°C. Aliquots of cDNA were further used for the subsequent quantitative polymerase chain reaction using Applied Biosystems via using the manufacture instructions. The target sequences were estimated using the primers specific to the corresponding cDNA.

Data analysis

Results are expressed as mean ± standard error of the mean. For the data analysis, Graphpad Prism software (5.02) (Trial Version), (San Diago, CA, USA) was used. One-way analysis of variance (ANOVA) was used for the statistical analysis via using the Dunnet's test. $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically significant, more significant, and most significant.

RESULTS AND DISCUSSION

Clinical studies suggest that the overweight during adolescence and childhood considerably related to dyslipidemia, increased blood pressure (BP), and insulin resistance in young adulthood.[30] Diabetes mellitus especially type II is strongly linked with cardiovascular risk and obesity.[31,32] Researches suggest that the diabetes induced in rodents, fed with the HFD produces a metabolic syndrome categorized via central obesity, diabetes mellitus-2, and insulin resistance dyslipidemia.[33,34] Diabetic rats were fed with the HFD show the progression of typical histopathological microvesicular steatosis escorted via an increment in hepatic cholesterol and TGs and a reduction in hepatic extraction fraction.[35] As already confirm that the lipid and carbohydrate metabolism produce dyslipidemia, henceforth STZ induces type 2 diabetes + HFD is one of the best models for scrutinizing the anti-obesity potential of the tested drug in the diabetic rats.[36,37] During the HFD, the hepatic marker, oxidative stress, inflammation and cytokines were increased and reduced level of Nrf2 in the liver was observed. Dose-dependent treatment of adropin significantly ($P < 0.001$) down-regulated the hepatic marker, oxidative stress, inflammation, and cytokines and increased the Nrf2 level. Table 1 exhibits the effect of adropin on the various parameters of experimental rats. HFD-induced control rats showed increased food (21.34 ± 0.64 g/100 d/day) and water intake (51.04 ± 0.63 ml/100 g/day) as compared to normal control. Adropin reduced the food (18.09 ± 0.45, 16.84 ± 0.34, and 14.84 ± 0.85 g/100 d/day) and water intake (50.45 ± 0.43, 49.12 ± 0.68, and 46.04 ± 0.67 ml/100 g/day) at a dose level of 2.5, 5, and 10 mg/kg, respectively. A similar momentum food (15.03 ± 0.83 g/100 d/day) and water intake (43.45 ± 0.78 ml/100 g/day) effect were also observed in the glibenclamide treated group rats.

Studies suggest that obesity and metabolic syndrome are linked with steatosis.[38] Consequently, experimental rodents developed the steatosis after feeding the HFD and experimental rats demonstrate a spontaneous polygenic form of diabetes and a commonly used model for diabetes.[39] Peripheral insulin resistance and increased blood glucose are the common features of the experimental model.[40,41] They revealed the mild hyperglycemia during fastening and enhanced gluconeogenesis. Studies suggest that chronic high-fat feed increase diabetes and its complications, boost dyslipidemia and increases the TG in the hepatic tissue leading the steatosis.[42,43] Table 1 demonstrates the effect of adropin on the experimental rats. Normal control rats showed the body weight 176.77 ± 4.34 g at the end of the experimental study. HFD control group rats exhibited reduced body weight 146.34 ± 3.46 g in comparison to control group rats. Adropin significantly ($P < 0.001$) increased the body weight 150.34 ± 4.32, 165.04 ± 2.78, and 173.68 ± 3.27 g at a dose level of 2.5, 5, and 10 mg/kg, respectively. Adropin (10 mg/kg) showed the increased body weight almost near to the normal control. On the contrary, glibenclamide significantly ($P < 0.001$) increased the body weight 171.43 ± 3.45 g at the end of the experimental study. During the HFD-induced obesity, the lipid profile altered due to the disease. Table 1 shows the effect of adropin and glibenclamide on the lipid profile of experimental rats. HFD control rats exhibited the altered level of lipid profile such as total cholesterol (TC) (212.98 ± 5.89 mg/dl), TG (250.53 ± 6.54 mg/dl), high-density lipoprotein-cholesterol (HDL-C) (18.93 ± 1.98 mg/dl), low-density lipoprotein-cholesterol (LDL-C) (144.35 ± 5.48 mg/dl), and very low-density lipoprotein (VLDL) (50.09 ± 2.12 mg/dl) as compared to normal control TC (71.76 ± 3.24 mg/dl), TG (68.35 ± 2.08 mg/dl), HDL-C (42.54 ± 2.09 mg/dl), LDL-C (15.54 ± 1.84 mg/dl), and VLDL (13.67 ± 1.83 mg/dl). Adropin significantly ($P < 0.001$) modulated the level of lipid parameters such as TC (80.45 ± 4.83 mg/dl), TG (97.5 ± 4.45 mg/dl), HDL-C (38.74 ± 1.38 mg/dl), LDL-C (22.21 ± 2.89 mg/dl), and VLDL (19.5 ± 1.73 mg/dl) as compared to HFD control. A similar effect was observed in the glibenclamide group rats TC (84.53 ± 3.84 mg/dl), TG (104.5 ± 5.43 mg/dl), HDL-C (36.78 ± 1.48 mg/dl), LDL-C (26.84 ± 1.93 mg/dl), and VLDL (20.9 ± 1.86 mg/dl). The altered level of BP was observed during the HFD-induced obesity. Normal control group rats showed the systolic BP (120.3 ± 4.04 mm Hg), diastolic BP (96.54 ± 3.12 mm Hg), mean BP (102.34 ± 3.21 mm Hg), and heart rate (444.64 ± 8.74 BPM). HFD control group rats showed the systolic BP (165.45 ± 3.94 mm Hg), diastolic BP (125.43 ± 2.83 mm Hg), mean BP (131.34 ± 2.83 mm Hg),

and heart rate (3647.6 ± 29.84 BPM). Adropin significantly ($P < 0.001$) altered the BP parameters viz., systolic BP (128.74 ± 4.09 mm Hg), diastolic BP (98.84 ± 3.94 mm Hg), mean BP (105.46 ± 2.78 mm Hg), and heart rate (380.5 ± 18.73 BPM). Glibenclamide significantly ($P < 0.001$) modulated the systolic BP (135.52 ± 5.03 mm Hg), diastolic BP (102.34 ± 2.34 mm Hg), mean BP (109.4 ± 3.45 mm Hg), and heart rate (420.61 ± 14.56 BPM) [Table 1].

Previous research suggests that during the HFD induced, enhancement were observed in the bodyweight increase, but some study showed the negative result.[44,45] A similar result was observed in this study, HFD-induced group demonstrated decreased body weight as comparison to other group rats. Adropin-treated group rats exhibited increased body weight as compared to HFD-treated group rats. Proinflammatory endocrine tissue like visceral adipose tissue may be responsible for enhancing the cardiometabolic risk that occurs as body mass index. Visceral adiposity, adiposity status is linked with an adverse cardiac profile such as insulin resistance, myocardial dysfunction and inflammation, all of the parameters are considered as the hallmark of obese phenotype.[46,47] Previous research suggests that lipid profile considerably increased and HDL-C, VLDL-C markedly increased and similar result was observed in the HFD group rats and adropin significantly ($P < 0.001$) reduced the TC, TG, LDL and increased the level of HDL, VLDL almost near to the normal group. HFD group rats showed a decreased level of insulin and improved level of leptin and adiponectin as compared to normal control. Normal Control (NC) group rats demonstrated the augmented level of insulin and decreased levels of leptin and adiponectin. Dose dependently treatment of adropin significantly ($P < 0.001$) increased the insulin level and decreased level

Table 1: The effect of adropin on the food intake, water intake, body weight and ratio, lipid profile, and blood pressure parameter in Wistar rats fed with high fat diet

Parameters	Groups					
	NC	HFD control	HFD + adropin (2.5 mg/kg)	HFD + adropin (5 mg/kg)	HFD + adropin (10 mg/kg)	HFD + glibenclamide (2.5 mg/kg)
Diet parameters						
Food intake (g/100 g/day)	14.54±0.73	21.34±0.64	18.09±0.45*	16.84±0.34**	14.84±0.85***	15.03±0.83***
Water intake (ml/100 g/day)	42.34±0.74	51.04±0.63	50.45±0.43ns	49.12±0.68*	46.04±0.67***	43.45±0.78***
Body weight						
Body weight (g)	176.77±4.34	146.34±3.46	150.34±4.32*	165.04±2.78**	173.68±3.27***	171.43±3.45***
Lipid parameters						
TC (mg/dl)	71.76±3.24	212.98±5.89	180.73±4.95**	139.74±5.43***	80.45±4.83***	84.53±3.84***
TG (mg/dl)	68.35±2.08	250.53±6.54	220.36±5.04**	150.4±4.35***	97.5±4.45***	104.5±5.43***
HDL-C (mg/dl)	42.54±2.09	18.93±1.98	20.45±1.89*	26.54±1.78***	38.74±1.38***	36.78±1.48***
LDL-C (mg/dl)	15.54±1.84	144.35±5.48	116.54±4.56**	83.12±0.68***	22.21±2.89***	26.84±1.93***
VLDL-C (mg/dl)	13.67±1.83	50.09±2.12	44.54±1.83**	30.08±2.03***	19.5±1.73***	20.9±1.86***
BP parameters						
Systolic BP (mm Hg)	120.3±4.04	165.45±3.94	160.87±4.09*	148.5±3.98***	128.74±4.09***	135.52±5.03***
Diastolic BP (mm Hg)	96.54±3.12	125.43±2.83	120.2±3.04*	114.45±2.83***	98.84±3.94***	102.34±2.34***
Mean BP (mm Hg)	102.34±3.21	131.34±2.83	129.34±2.65ns	121.37±3.12***	105.46±2.78***	109.4±3.45***
Heart rate (BPM)	444.64±8.74	3647.6±29.84	2193.5±25.48***	937.56±21.83***	380.5±18.73***	420.61±14.56***

Statistically significant different are indicated via asterisks and estimated via ANOVA. ***P<0.001; **P<0.001; *P<0.05 compared with the model group. ANOVA: Analysis of variance; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; VLDL-C: Very LDL-C; BP: Blood pressure; HFD: High fat diet; NC: Normal Control

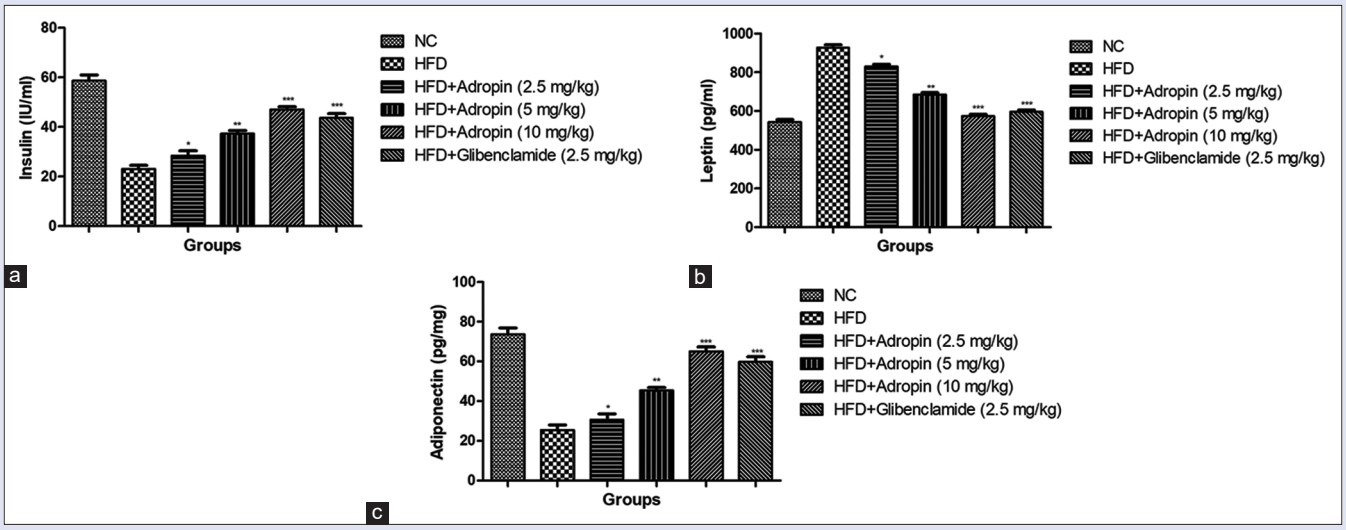

Figure 1: The effect of adropin on the plasma insulin, leptin and adiponectin in high fat diet/streptozotocin-induced obesity rats. (a) Insulin, (b) leptin and (c) adipnectin. Statistically significant different are indicated via asterisks and estimated via analysis of variance. ***$P < 0.001$, **$P < 0.001$, *$P < 0.05$ compared with the model group

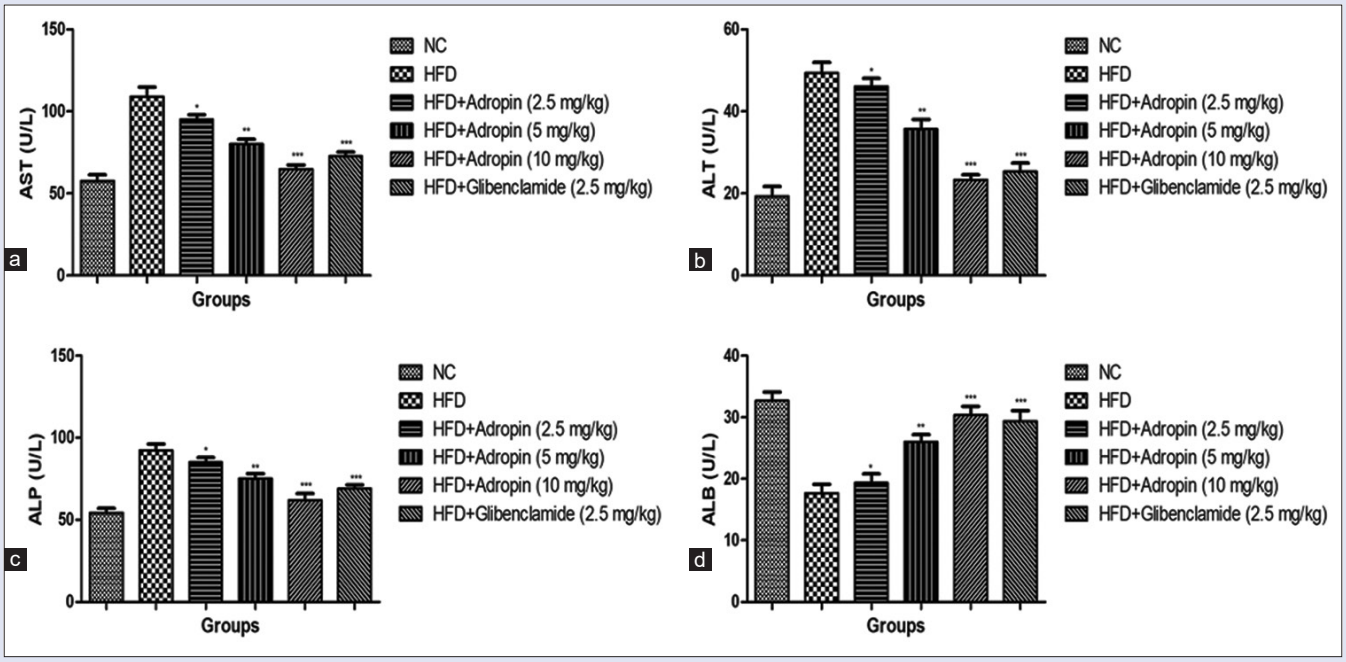

Figure 2: The effect of adropin on the hepatic parameters in high fat diet/streptozotocin -induced obesity rats. (a) Aspartate transaminase, (b) alanine transaminase, (c) Alkaline phosphotase and (d) Albumin. Statistically significant different are indicated via asterisks and estimated via analysis of variance. ***$P < 0.001$, **$P < 0.001$, *$P < 0.05$ compared with the model group

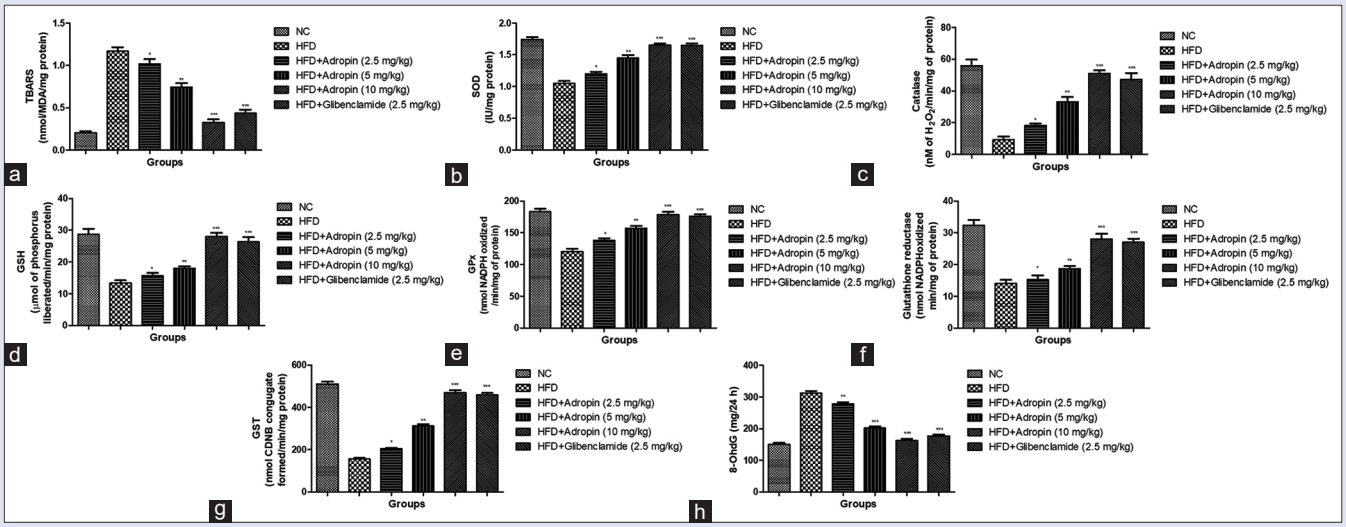

Figure 3: The effect of adropin on the antioxidant parameters in high fat diet/streptozotocin-induced obesity rats. (a) Thiobarbituric acid reactive substances, (b) superoxide dismutase, (c) catalase, (d) glutathione, (e) glutathione peroxidase, (f) glutathione reducatase, (g) glutathione S-transferase and (h) 8-OhdG. Statistically significant different are indicated via asterisks and estimated via analysis of variance. ***$P < 0.001$, **$P < 0.001$, *$P < 0.05$ compared with the model group

of adiponectin and leptin as compared to the HFD group. A similar momentum was observed in the Gli group [Figure 1].

During the HFD-induced obesity, altered level of hepatic enzymes was observed. A similar result was found in the HFD control group rats. HFD control group rats showed the down-regulation in the level of AST, ALT, ALP, and upregulation of ALB were also observed and dose dependently treatment of adropin significantly ($P < 0.001$) and glibenclamide-treated group exhibited the increased ALT, AST, ALP level, and decreased ALB level [Figure 2]. During the expansion of obesity, antioxidant enzymes were also altered. HFD-induced obesity rats showed the upregulated the TBARS, 8-OhdG level, and downregulated the SOD, GPx, GSH, GR, GST, and dose dependently administration of adropin significantly ($P < 0.001$) reduced the level of TBARS, 8-OhdG, and increased level of SOD, GPx, SOD, GSH, GR, and GST [Figure 3]. Oxidative stress plays an important role in the expansion of various diseases such as hyperlipidemia, diabetes, and many more. In the etiology of hepatic damage, oxidative stress plays a key role.[4] In the current experimental study, we scrutinize the antioxidant potential of adropin against the HFD induced diabetic rats.

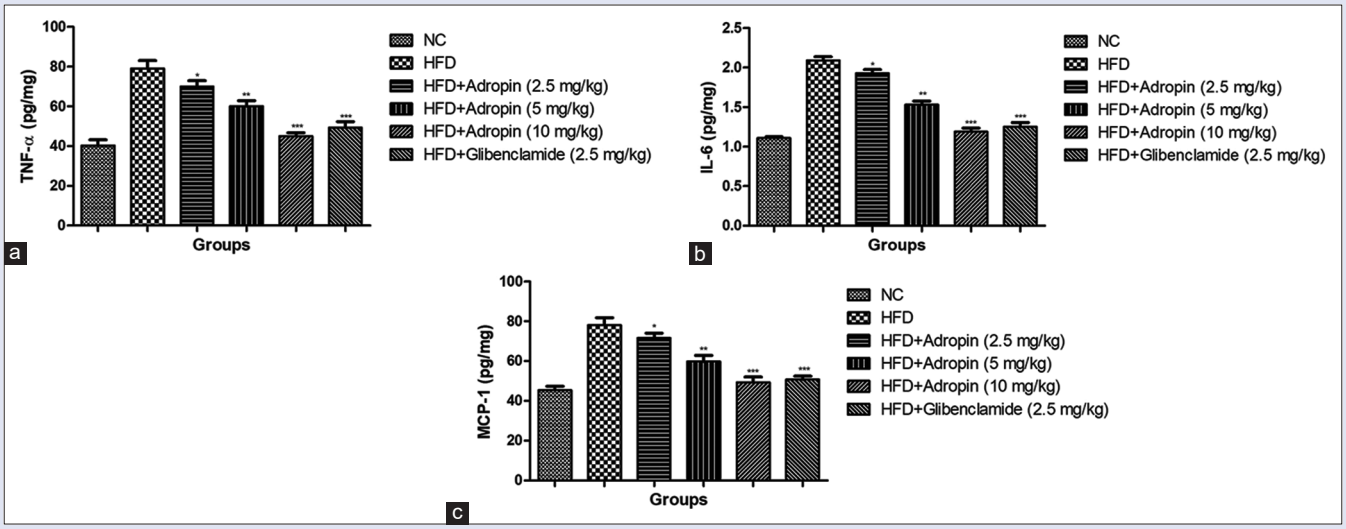

Figure 4: The effect of adropin on the cytokines in high fat diet/streptozotocin-induced obesity rats. (a) Tumor necrosis factor-α, (b) interleukin-6, and (c) monocyte chemoattractant protein-1. Statistically significant different are indicated via asterisks and estimated via analysis of variance. ***$P < 0.001$, **$P < 0.001$, *$P < 0.05$ compared with the model group

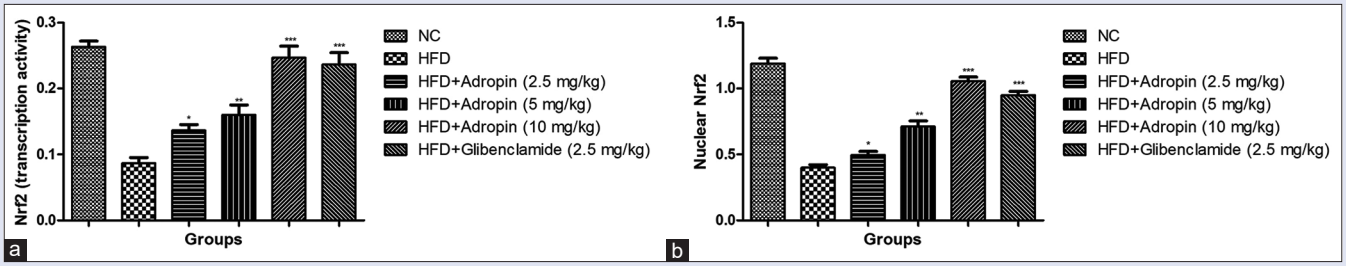

Figure 5: The effect of adropin on the nuclear factor erythroid 2-related factor 2 expression in high fat diet/streptozotocin-induced obesity rats. Statistically significant different are indicated via asterisks and estimated via analysis of variance. ***$P < 0.001$, **$P < 0.001$, *$P < 0.05$ compared with the model group. (a) Nrf2 (transcription activity) and (b) Nuclear Nrf2

We may conclude that adropin demonstrated the antioxidant potential by altering the endogenous parameters based on the findings. Adropin enhanced the liver transaminases and increased the scavenging of free radical, enhanced endogenous antioxidant enzymes activity leading to the down-regulation of the inflammatory reaction, oxidative stress, decreased the fibrotic process, reduced the DNA injury and augmented the lipid metabolism. Adropin can also activate the protein and alter the redox status of cells. The current process affects the transcriptional reaction and cell signaling elaborates the lipid and glucose metabolism.

HFD-induced obesity group rats demonstrated the increased level of IL-6, monocyte chemoattractant protein-1 (MCP-1) and TNF-α, confirm the enhancement of inflammation disease. Adropin significantly ($P < 0.001$) downregulated the level of IL-6, MCP-1, and TNF-α as compared to HFD control group rats. Adropin (10 mg/kg) exhibited the reduced level of TNF-α, IL-6, and MCP-1 almost near to the normal control. Glibenclamide treated group rats showed the decreased level of TNF-α, IL-6, and MCP-1 [Figure 4]. Previous reports indicate that chronic low-grade inflammation of the adipose tissue plays a crucial role in the development of HFD-induced insulin resistance and obesity.[48,49] The anti-inflammatory and proinflammatory cytokines secreted from the adipose tissue are called as adipokines and suggest the direct link between systemic inflammation and obesity.[3,48] Research suggests that during the obesity, upregulation in the expression of pro-inflammatory adipokines such as IL-6, MCP-1, and TNF-α and also decreased the expression of adiponectin.[50,51] While fed the HFD, there was enhancement in the systemic leptin along with the inflammation, infection upregulation, and increased the production of cytokines.[52] On the basis of the result, we can hypothesize that adropin significantly ($P < 0.001$) downregulated the inflammation in the adipose tissue induced via HFD. Adropin significantly reduced the proinflammatory cytokines, namely IL-6, MCP-1, and TNF-α as well as leptin.

In the current experimental study, we determined the level of hepatic Nrf2 level. HFD group rats exhibited the reduced level of Nrf2 transcription activity and nuclear Nrf2 level and dose dependently treatment of adropin and glibenclamide significantly ($P < 0.001$) increased the level of transcription activity and nuclear Nrf2 level [Figure 5]. In the current study, we observed the reduced production of Nrf2 in the hepatic tissue in rats fed with HFD, which results in a reduction in GSH levels, which is reversible with adropin care. Several studies suggest that the regeneration and *de novo* synthesis of GSH, activating the Nrf2 transcriptional factor.[53] In hepatocytes, oxidative stress boosts the Nrf2 transcriptional factor and dose dependently treatment of adropin produce the protection against lipoapoptosis.[54,55] It is well known that Nrf2 regulates the different proteins involved in fatty acids and other lipids metabolism and synthesis. Previous studies suggest that the level of Nrf2 reduced in the aged rats.[56-58] Furthermore, the rats received the adropin significantly enhanced the GSH level and glutamine cysteine ligase activity in the liver via the involvement of Nrf2. Previous literature suggests that the Nrf2 involved in the lipid deposition in the hepatic tissue.[59,60] The Nrf2

expression and activation are decreased in the hepatic tissue and dose dependently treatment increased the expression of Nrf2.

CONCLUSION

In the current experimental study, the authors can conclude that adropin increased insulin sensitivity and reduced the blood glucose level. Adropin significantly ($P < 0.001$) reduced the lipid profile and increase the level of HDL. Adropin significantly ($P < 0.001$) decreased the lipogenesis in the adipose tissue and also reduced the inflammatory reaction via minimizing the cytokines level. Finally, adropin altered the Nrf2 pathway via anti-inflammatory and antioxidant mechanism.

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Conflicts of interest

There are no conflicts of interest.

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