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Pyrroloquinoline quinone attenuates obesity associated low grade inflammation

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ABSTRACT

Aim: To investigate the impact of pyrroloquinoline quinone (PQQ) *per se* and in combination with atorvastatin (ATS) in a model of high fat, 10% fructose diet (HFFD) induced obesity associated low-grade inflammation.

Methods: Rats were either fed with HFFD or normal rat chow for 10 weeks and subsequently administered with ATS, PQQ 10 and 20 mg/kg, *p.o per se* and in combination of respective doses of 10 and 20 mg/kg, *p.o* daily for five weeks. The effect of ATS and PQQ on blood glucose, lipid profile, serum insulin, CRP, TNF-α, IL-1β, IL-18, IL-6, adiponectin and histopathological examination were investigated.

Results: Compared to the positive control, rats receiving PQQ and ATS revealed significant decrease in body weights and anthropometric parameters. The rats receiving PQQ *per se* and along with ATS exhibited improved glucose tolerance, lipid profile, insulin indices and displayed lower serum levels of CRP, inflammatory cytokines TNF-α, IL-1β, IL-18 and IL-6 along with a rise in adiponectin. Histopathological evaluation revealed, decrease in the adipocyte number with infiltration of inflammatory cells in the positive control rats which was not evident in the adipose tissue of the treated rats.

Conclusion: Treatment with ATS along with PQQ more effectively attenuated low-grade inflammatory response and hyperlipidaemia in HFFD induced obese rats.

1. Introduction

Obesity is a pandemic health concern and is strongly associated with cluster of diseases caused by long term energy imbalance. Excess nutrition/diet and sedentary lifestyle leads to the development of excessive lipid accumulation in adipose and peripheral tissues leading in elevated blood pressure, hyperlipidaemia, insulin resistance and chronic low grade inflammation, contributing to systemic metabolic dysfunction (Jung et al., 2014). Inflammation plays a key role in the manifestation of the comorbidities of obesity and is the product of dysregulated adipose tissue. Obesity is the condition of fat induced prolonged inflammation that causes decrease in immune defence (Magnuson et al., 2015).

Adipose tissue normally involves different immune cells that support and maintain the integrity of adipocytes and hormonal sensitivity together. Macrophages experience dramatic changes during obesity that demonstrate a more proinflammatory phenotype and secrete cytokines such as TNF-α, IL-1β, IL-8, IL-6, IFN-γ, leptin and resistin. The production of these molecules coupled with the destruction of adipocytes, triggers chronic inflammation, affects other systems by modifying their functions, leading to the development of insulin resistance and metabolic diseases (Saltiel and Olefsky, 2017)(Castro et al., 2017). It has lately been shown that adipocyte hypoxia stimulates cytokine activity and strongly contributes to the growth and development of inflammation linked to obesity (Castro et al., 2017). IL-1β, a major cytokine produced largely by macrophages, is involved in the development of obesity associated with insulin resistance. Growing evidence indicates that IL-1β is critically engaged in translating obesity-associated inflammation into insulin resistance in rodent models and has been involved in mediating the inflammatory and catabolic effects of macrophages on adipose cells (Caër et al., 2017)(Bing, 2015). In turn IL-1β directly stimulates the production of cytokines such as IL-6 (Fuggetta et al., 2019). Another cytokine IL-6, produced prominently by pro-inflammatory macrophages, is considered to change insulin signals and enhance inflammation in murine adipocytes (Caër et al., 2017). IL-18 is produced in many distinct kinds of cells such as macrophages, endothelial cells, vascular smooth muscle cells, dendritic cells and Kupffer cells, and was observed constitutively and strongly associated with metabolic syndrome and its implications. It has been revealed that circulating concentrations of IL-18 was found to be elevated in subjects with metabolic syndrome and is useful for anticipating cardiovascular events (Trøseid et al., 2010).

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Hyperlipidaemia including both hypercholesterolemia and hypertriglyceridemia are the hallmark of obese states. Currently one of the main class of anti-hyperlipidaemic drugs is hydroxymethylglutaryl-coenzyme A reductase inhibitors which includes the statins. Clinical studies conducted on statins viz., GREACE (Athyros et al., 2004), JUPITER (Ridker and Silvertown, 2008), MIRACL (Kinlay et al., 2008) and ARMYDA (Pristipino et al., 2004) trial have revealed that they express anti-inflammatory action which contributes in reducing cardiovascular events (Athyros et al., 2018). However, the treatment with statins have clinical limitations due to various reasons including unsatisfactory treatment outcomes (Barry et al., 2018). As many as 20% of individuals with clinical indications of statin therapy are unable to take statin because of some degree of intolerance and 40–75% of patients discontinue their statin therapy within 1–2 years after initiation (Toth et al., 2018). According to mancini et al., about 70–80% of statin-treated patients are intolerant to treatment, and 20–30% are suspected to be statin intolerant (Mancini et al., 2016). A systematic review that used data from 1.9 million patients indicated that high dose of statins therapy increases the risk of new onset of diabetes mellitus and also deteriorates the glycaemic control in patients with known diabetes mellitus (Angelidi et al., 2018). It is estimated that between 7% and 29% of patients taking statins experience some type of muscle-associated toxicity (Barry et al., 2018). The most frequently mooted and widely researched Statin induced Coenzyme Q10 (CoQ10)/ubiquinone deficiency is associated with its muscle centric side effects like myalgia (Ramachandran and Wierzbicki, 2017). It is generally recognised that CoQ10 supplementation reverses the ubiquinone deficiency and mitochondrial dysfunction (Deichmann et al., 2010)(Choi et al., 2016). Obesity contributes to numerous modifications in the body owing to surplus nutrients supply that can overwhelm the Kerb's cycle and mitochondrial respiratory chain, causing a mitochondrial dysfunction leading to a greater ROS generation that may exacerbate the inflammatory process (de Mello et al., 2018).

Based on the current knowledge on obesity-inflammation phenomenon, the role of mitochondrial dysfunction in mediating downstream rise in inflammatory cascade. We thought it prudent to explore the impact of pyrroloquinoline quinone (PQQ), a ubiquitous natural bacterial redox cofactor ("GRAS Notice for a Pyrroloquinoline Quinone (PQQ) Disodium Salt," 2017) in abating obesity associated low-grade inflammation in a pre-clinical model of HFFD fed obesity and associated low grade inflammation. PQQ in the reduced form, its aroxyl radical-scavenging activity is 7.4-fold higher than that of vitamin C, the most active water-soluble antioxidant (Jonscher et al., 2017)(Akagawa et al., 2016). PQQ in combination with quinoprotein dehydrogenases oxidizes numerous substrates in the periplasm coupled to respiratory chains and regulates the cellular growth and differentiation in mammalian systems (Wan et al., 2017). PQQ stimulates mitochondrial biogenesis *in vitro* by activating peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α, an important regulator of metabolism and mitochondrial oxidative defence (Chowanadisai et al., 2010). It is known to increase the expression and activity of SIRT1/PGC1α in NIH/3T3 cells (Saihara et al., 2017). The major source of this important antioxidant in mammals is diet (Rucker et al., 2009). PQQ is known to be highly enriched in human breast milk (Mitchell et al., 1999), mediating prevention of excess maternal obesity-induced oxidative stress. However, the impact of PQQ in the paradigm of obesity associated low grade inflammation remains unexplored. Hence the current preclinical study investigated the impact of PQQ on obesity and associated low grade inflammation. We expanded our protocol to decipher the impact of PQQ supplementation on clinical outcomes of atorvastatin (ATS) by profiling anthropometric, metabolic and inflammatory markers in a model of diet induced obesity in rats.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats, 180–200 g were obtained from Bharat Serums and Vaccines Limited, Thane, India. On arrival, rats were kept in an environmentally controlled room temperature 25 ± 2 °C, humidity 65 ± 5%, 12-h dark–light cycle. All rats were housed in polypropylene cages with corn cobb as bedding and were freely access to water and standard pelleted laboratory animal diet. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), protocol no. CPCSEA-BCP/2016-02/12 and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.2. Materials and sources

Atorvastatin was obtained from Enaltec Labs Pvt. Ltd., Navi Mumbai, India. Pyrroloquinoline Quinone was obtained from Wuxi Cima Science Co. Ltd., Wuxi Jiangsu, China. Cholesterol was obtained from Fermenta Biotech Ltd., Thane, India. All other chemicals and reagents obtained from local suppliers were of analytical grade.

2.3. Drug treatments in HFFD obesity model

After one week of acclimatisation, rats were divided into eight groups of 6 rats in each group. The rats of group I, control rats, had free access to normal rat chow consisting of carbohydrate (52%), proteins (16.65%), fats (3.72%), dietary fibre (5.5%) and fresh water *ad libitum* throughout the study. The rats of the other seven groups were fed with High Fat, 10% Fructose Diet (HFFD) consisting of carbohydrate (56.04%), proteins (12.36%), fats (24.44%), dietary fibre (4.70%) and cholesterol (1%). 10% fructose solution was prepared in fresh water. The energy provided by the normal diet and high fat diet was 3020 kcal/kg and 4935 kcal/kg respectively, whereas that provided by fructose was 1300 kcal/kg. Therefore, total energy provided by HFFD was 6235 kcal/kg. At the end of 10th week body weight, anthropometric parameter and fasting blood glucose level were captured to confirm induction of obesity and subsequently were randomized for treatment as follows: group II, positive control; group III, atorvastatin 10 mg/kg, *p.o.* (ATS 10); group IV, ATS 20 mg/kg, *p.o.* (ATS 20); group V, PQQ 10 mg/kg, *p.o.* (PQQ 10); group VI, PQQ 20 mg/kg, *p.o.* (PQQ 20); group VII, ATS 10 + PQQ 10 mg/kg, *p.o.*; group VIII, ATS 20 + PQQ 20 mg/kg, *p.o.* Daily for 5 weeks. Dose selection for PQQ was done based on the earlier reports mentioned in GRAS notice of PQQ (Cheng, 2017).

2.4. Body weight, dietary and anthropometrical parameters

Body weights of the animals were measured once a week. Food and water intake of various groups were monitored throughout the study and used to calculate the average caloric intake and feed efficiency ratio (Salaj et al., 2013). The body mass index (BMI), lee's index, and the abdominal circumferences to thoracic circumferences (AC/TC) ratio were also determined (Novelli et al., 2007).

2.5. Serum metabolic parameters

On 99th day, rats were fasted overnight. Oral glucose tolerance test was performed on 100th day. The glucometer (Dr. Morepen® glucose monitor) was utilised to determine the glucose concentration. On 101th day, rats were euthanized using dry CO_2; blood was collected from retro-orbital plexus and rats were euthanized in CO_2 Chamber. Blood samples were then centrifuged at 3000 × *g* for 15 min at 4 °C. Serum was aliquoted and stored at −20 °C for various biochemical assays. The

liver, spleen and intra-abdominal fat of all animals were dissected and weighed. Various biochemical and IAF histopathological studies were carried out.

Quantitative estimation of serum total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), Very low-density lipoprotein cholesterol (VLDL-C) and non-HDL-C was done using commercial diagnostic kits (Erba Diagnostics, Mannheim, Germany). Estimation of fasting serum insulin was performed using Enzyme Linked Immuno Sorbent Assay (ELISA) kit (Elabscience®, Houstan, Texas). Insulin sensitivity indices such as homeostatic model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were also calculated (Singh, 2010).

The concentrations of TNF-α, IL-1β, IL-6, IL-18, CRP were determined using ELISA kit (Raybiotech® Norcross, GA) and adiponectin was determined using ELISA kit (Bioassay technology laboratory shanghai, China) according to the manufacture's protocol.

2.6. Histopathological studies

The intra-abdominal adipose tissue specimens were dehydrated with increasing grades of the alcohol, i.e. 70, 80, 90% and cleared with xylene followed by embedding in paraffin. Thereafter, the processed tissue sections were sectioned (at 5 μm) and taken on clean glass slides, stained with haematoxylin and eosin (H&E) and observed under the microscope at 400X magnification. The intra-abdominal fat was analysed for the size and number of adipocytes in five different fields, and the average number of adipocytes per field was determined.

2.7. Statistical analysis

All results are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA). The differences among the experimental groups were analysed with one-way analysis of variance, followed by Dunnett's multiple comparison test as a *post-hoc* analysis. For OGTT data, two-way analysis of variance (with time and treatment as variables) followed by Tukey's multiple comparison test as a *post-hoc* analysis was used. Statistical significance was considered at $^{****}P < 0.0001$; $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ and $^{\dagger\dagger\dagger\dagger}P < 0.0001$; $^{\dagger\dagger\dagger}P < 0.001$; $^{\dagger\dagger}P < 0.01$; $^{\dagger}P < 0.05$ when compared with positive control and normal control groups respectively.

3. Results

3.1. Effect of PQQ and ATS on body weight, dietary and anthropometrical parameters

Consumption of HFFD resulted in significant increase in average body weight, feed efficiency ratio, food intake, water intake, BMI, lee's index and AC/TC ratio when compared with the normal control. Treatment with ATS (10 and 20 mg/kg), PQQ (10 and 20 mg/kg) *per se* and the combination of ATS 10 + PQQ 10 mg/kg, ATS 20 + PQQ 20 mg/kg significantly prevented this HFFD induced rise in body weight, food intake, water intake and anthropometrical parameters. However, treatment with ATS, PQQ *per se* and in combination did not result in any significant difference in average caloric intake (Table 1 and Table 2).

3.2. Effect of PQQ and ATS on organ weights and intra-abdominal fat

Consumption of HFFD resulted in significant increase in liver, spleen and IAF when compared with normal control group. Treatment with ATS, PQQ *per se* and in combination therapy significantly reversed the above alterations in organ weights and fat depositions (Table 3).

3.3. Effect of PQQ and ATS on blood glucose concentration during oral glucose tolerance test

Positive control rats showed significant decrease in glucose tolerance when compared to normal control. After 180 min of post glucose load, the average glucose concentration of positive control rats was 146.8 mg/dL compared to 93.3 mg/dL for the normal control. This indicates an impaired glucose tolerance due to HFFD in the rats, which is a forerunner to T2DM. Treatment with ATS, PQQ *per se* significantly prevented the glucose intolerance. Further, the improved glucose tolerance was more pronounced in the groups treated with ATS 10 + PQQ 10 mg/kg (97.6 mg/dL), ATS 20 + PQQ 20 mg/kg (94.83 mg/dL) (Fig 1).

3.4. Effect of PQQ and ATS on fasting serum insulin and insulin sensitivity indices

Consumption of HFFD resulted in significant increase in serum insulin levels when compared with normal control group indicating hyperinsulinemia. Treatment with ATS (10 and 20 mg/kg) alone did not showed any significant difference while PQQ *per se* and in combination with ATS showed significant decrease in serum insulin levels when compared with positive control rats. HOMA-IR, an index of insulin resistance was significantly higher whereas QUICKI was significantly lower in positive control rats when compared with normal control. PQQ *per se* and in combination with ATS countered the hyperinsulinemia which can be attributed to decrease in insulin resistance. However, ATS *per se* failed to prevent the deflections in the serum insulin levels and QUICKI (Table 4).

3.5. Effect of PQQ and ATS on serum lipid profile and atherogenic index

Obesity induced by HFFD resulted in a significant increase in serum total cholesterol, triglycerides, LDL-C, VLDL-C, non-HDL-C, mean atherogenic index in the positive control rats. Whereas HDL-C was significantly reduced vis-à-vis normal control values. The perturbations in the lipid profile were prevented by ATS, PQQ *per se* and the combination therapy (Table 5).

3.6. Effect of PQQ and ATS on serum cytokines and CRP

Consumption of HFFD led to significantly increase in the protein concentration of serum TNF-α, IL-1β, IL-6, IL-18, CRP whereas, decrease in the level of serum adiponectin when compared with normal control. Treatment with ATS, PQQ *per se* and the combination therapy significantly decreased the level of TNF-α, IL-1β, IL-6, IL-18, CRP and increased the levels of adiponectin when compared to positive control. Combination therapy showed greater therapeutic efficacy than ATS, PQQ *per se* indicating attenuated markers of inflammation (Fig. 2).

3.7. Effect of PQQ and ATS on histology of adipose tissue

The number of adipocytes per high-power of field (HPF) in the intraabdominal fat were significantly decreased in the positive control group when compared with normal control. The number of adipocytes per HPF was increased significantly in the ATS, PQQ *per se* and the combination therapy groups, indicative of decrease in the size of adipocytes reflecting reduced accumulation of fat (Fig. 3) (Table 3).

4. Discussion

In the present study, we have investigated the effect of PQQ on obesity associated chronic low-grade inflammation in HFFD fed SD rats. Effect of PQQ and atorvastatin were investigated on body weight, food intake, water intake, calorie intake and feed efficiency ratio. In the beginning of the study, for the first 3 weeks, food intake and water

Table 1
Effect of PQQ and ATS on body weight and dietary intake.

	Body weight (gm)	Food intake (gm)	Water intake (mL)	Calorie intake (kcal/g/day)	Feed efficiency ratio
Normal control	344.66 ± 3.92	16.67 ± 0.07	36.04 ± 0.17	75.9 ± 0.08	18.2 ± 0.33
Positive control (HFFD)	512.33 ± 36.2††††	17.15 ± 0.11†	38.42 ± 0.43††††	118 ± 1.44††††	19.76 ± 0.45†
ATS 10	337 ± 3.07****	16.45 ± 0.13***	35.81 ± 0.07****	116.3 ± 0.0††††	17.47 ± 0.34***
ATS 20	340 ± 10.67 ****	16.35 ± 0.17****	36.12 ± 0.04****	118.4 ± 0.0††††	17.26 ± 0.42***
PQQ 10	348.33 ± 15.62****	16.42 ± 0.05***	36.84 ± 0.13**	116.7 ± 0.05††††	17.5 ± 0.44***
PQQ 20	326.5 ± 2.89 ****	16.64 ± 0.05**	35.78 ± 0.55****	117.4 ± 0.0††††	16.83 ± 0.23****
ATS10 + PQQ10	342.5 ± 4.12 ****	16.41 ± 0.053****	36.53 ± 0.40***	121.1 ± 0.0††††	17.9 ± 0.35**
ATS20 + PQQ20	342.83 ± 4.61****	16.84 ± 0.12	36.99 ± 0.01**	119.9 ± 0.0††††	17.57 ± 0.23***

Values are as mean ± SEM (n = 6 for all groups). Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test as a *post-hoc* analysis to identify significant differences among the groups.
HFFD, High fat, 10% fructose diet; ATS 10. Atorvastatin 10 mg/kg; ATS 20. Atorvastatin 20 mg/kg; PQQ 10, Pyrroloquinoline quinone 10 mg/kg; PQQ 20, Pyrroloquinoline quinone 20 mg/kg; ATS 10 + PQQ 10, Atorvastatin + Pyrroloquinoline quinone 10 mg/kg; ATS 20 + PQQ 20, Atorvastatin 20 + Pyrroloquinoline quinone 20 mg/kg.
$^{††††}P < 0.0001$; $^{†††}P < 0.001$; $^{†}P < 0.05$ when compared to the control group.
$****P < 0.0001$; $***P < 0.001$; $**P < 0.01$; $*P < 0.05$ when compared with positive control group.

Table 2
Effect of PQQ and ATS on anthropometrical parameters.

	Body mass index (g/cm^3)	Lee's index (g/cm$^{1/3}$)	AC/TC ratio
Normal control	0.74 ± 0.02	0.32 ± 0.005	1.077 ± 0.009
Positive control (HFFD)	0.92 ± 0.08††	0.33 ± 0.010	1.19 ± 0.01††
ATS 10	0.67 ± 0.01****	0.31 ± 0.002**	1.08 ± 0.02**
ATS 20	0.66 ± 0.02****	0.30 ± 0.005**	1.05 ± 0.008***
PQQ 10	0.70 ± 0.02***	0.31 ± 0.004*	1.05 ± 0.008***
PQQ 20	0.63 ± 0.01****	0.30 ± 0.004***	1.08 ± 0.02**
ATS10 + PQQ10	0.65 ± 0.01****	0.30 ± 0.003**	1.06 ± 0.03***
ATS20 + PQQ20	0.66 ± 0.01****	0.30 ± 0.002**	1.09 ± 0.02**

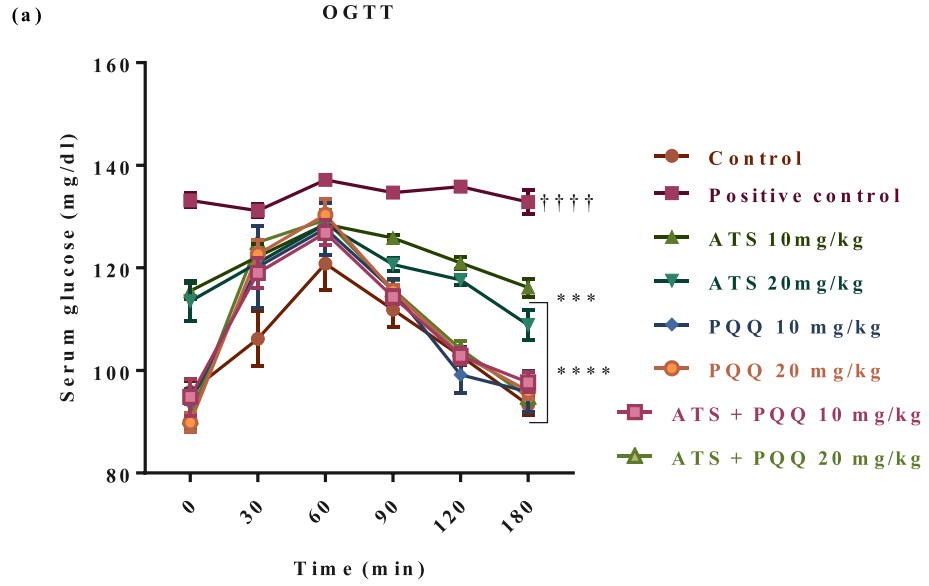
Values are as mean ± SEM (n = 6 for all groups). Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test as a *post-hoc* analysis to identify significant differences among the groups.
HFFD, High fat, 10% fructose diet; ATS 10. Atorvastatin 10 mg/kg; ATS 20. Atorvastatin 20 mg/kg; PQQ 10, Pyrroloquinoline quinone 10 mg/kg; PQQ 20, Pyrroloquinoline quinone 20 mg/kg; ATS 10 + PQQ 10, Atorvastatin + Pyrroloquinoline quinone 10 mg/kg; ATS 20 + PQQ 20, Atorvastatin 20 + Pyrroloquinoline quinone 20 mg/kg.
$^{††}P < 0.01$ when compared to the control group.
$****P < 0.0001$; $***P < 0.001$; $**P < 0.01$; $*P < 0.05$ when compared with positive control group.

Table 3
Effect of PQQ and ATS on organ weights and intra-abdominal fat.

	Liver weight (gm)	Spleen weight (gm)	Intra-abdominal fat (gm)	Adipocyte number
Normal control	10.82 ± 0.32	0.65 ± 0.02	4.57 ± 0.31	17
Positive control (HFFD)	13.4 ± 0.45†††	1.08 ± 0.02††††	10.18 ± 0.16††††	10
ATS 10	10.37 ± 0.44****	0.72 ± 0.02****	3.99 ± 0.44****	12.20
ATS 20	10.78 ± 0.39****	0.7 ± 0.03****	3.775 ± 0.26****	13.11
PQQ 10	11.5 ± 0.29**	0.66 ± 0.02****	4.95 ± 0.30****	13.15
PQQ 20	11.4 ± 0.22**	0.66 ± 0.02****	4.06 ± 0.36****	12.60
ATS10 + PQQ10	11.13 ± 0.31**	0.66 ± 0.02****	4.42 ± 0.46****	14
ATS20 + PQQ20	10.36 ± 0.30****	0.66 ± 0.02****	4.4 ± 0.29****	15

Values are as mean ± SEM (n = 6 for all groups). Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test as a *post-hoc* analysis to identify significant differences among the groups.
HFFD, High fat, 10% fructose diet; ATS 10. Atorvastatin 10 mg/kg; ATS 20. Atorvastatin 20 mg/kg; PQQ 10, Pyrroloquinoline quinone 10 mg/kg; PQQ 20, Pyrroloquinoline quinone 20 mg/kg; ATS 10 + PQQ 10, Atorvastatin + Pyrroloquinoline quinone 10 mg/kg; ATS 20 + PQQ 20, Atorvastatin 20 + Pyrroloquinoline quinone 20 mg/kg.
$^{††††}P < 0.0001$; $^{†††}P < 0.001$when compared to the control group.
$****P < 0.0001$; $**P < 0.01$ when compared with positive control group.

intake were found to be less in all the groups fed with HFFD than that of control group. After 3 weeks gradual increase in the intake of food and water was observed which resulted in high consumption of calories and increased body weight. Treatment with ATS or PQQ or both countered the rising body weight, food intake, water intake and feed efficiency ratio. Anthropometrical index was investigated to determine the threshold for obesity and to predict its adverse effects on lipid profile in rats (Novelli et al., 2007). BMI, Lee's index and AC/TC ratios were found to be significantly decreased in all the treatment groups as compared to positive control rats. These indices proved the induction of obesity. The investigation disclosed significant hypoglycaemic impact of PQQ at the 15th week, together with marginal hypoglycaemic effect at all other time points, as stated by fasting blood glucose level and OGTT. In the atorvastatin *per se* group, the magnitude of fasting blood glucose concentration and increase in oral glucose tolerance was less pronounced than that of PQQ *per se* and combination groups. Similar effect of declining in blood glucose concentrations and serum insulin levels was noted in previous studies in STZ induced diabetes mice



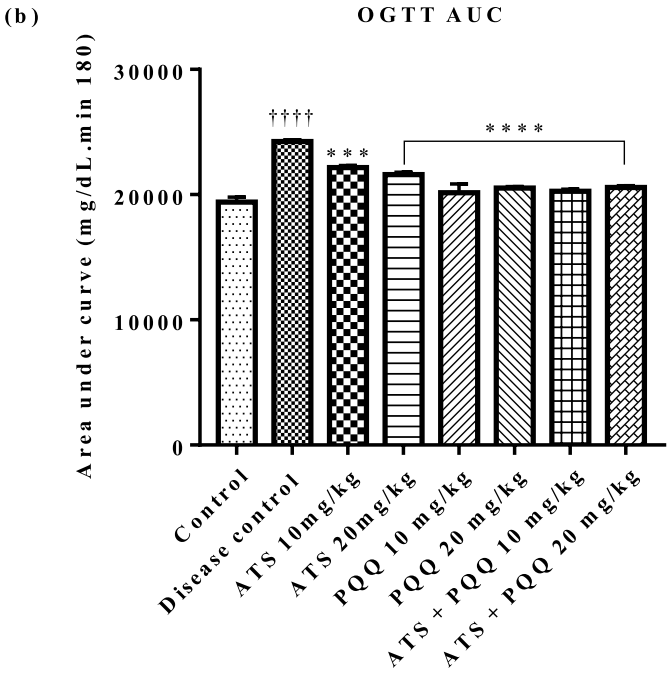


Fig. 1. Effect of PQQ and ATS on serum glucose concentration during (a) OGTT and (b) $AUC_{180\,min.}$ Values are as mean ± SEM (n = 6 for all groups). Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test as a *post-hoc* analysis to identify significant differences among the groups. HFFD, High fat, 10% fructose diet; ATS 10. Atorvastatin 10 mg/kg; ATS 20. Atorvastatin 20 mg/kg; PQQ 10, Pyrroloquinoline quinone 10 mg/kg; PQQ 20, Pyrroloquinoline quinone 20 mg/kg; ATS 10 + PQQ 10, Atorvastatin + Pyrroloquinoline quinone 10 mg/kg; ATS 20 + PQQ 20, Atorvastatin 20 + Pyrroloquinoline quinone 20 mg/kg. $^{††††}P < 0.0001$ when compared to the control group. $^{****}P < 0.0001$; $^{***}P < 0.001$ when compared with positive control group.

treated with PQQ (Kumar and Kar, 2015). Food and water consumption in HFFD fed rats gradually reduced after PQQ therapy in line with enhanced blood glucose concentrations and oral glucose tolerance. In contrast, positive control rats displayed noticeably increased serum fasting insulin levels indicating hyperinsulinemia. This hyperinsulinemia was further confirmed through associated parameters indicating significant reduction of QUICKI and increased HOMA-IR, an indices of insulin resistance in HFFD fed rats. Increased insulin sensitivity in ATS *per se* group was not observed which was pronounced in the PQQ *per se* and the PQQ + ATS treated groups of rats. The gentle impact of atorvastatin on insulin that we observed resonated with an earlier report (Kanda et al., 2003). However, after administration of atorvastatin we did not observe a reduction in insulin resistance as previously reported (Suzuki et al., 2005).

High TC, TG, VLDL-C, LDL-C and low HDL-C indicate disordered lipid metabolism and predisposition to cardiovascular disease (Ibitoye et al., 2017). These alterations could predispose the risk of developing atherosclerosis and cardiovascular diseases, while reduction in HDL cholesterol could intensify the development of atherosclerosis and cardiovascular diseases. In our findings PQQ *per se* and in combination was equally efficacious as compared to atorvastatin in significantly lowering the serum TC, triglyceride, LDL, VLDL and non-HDL in comparison with positive control rats. To provide a stronger evidence of predisposition to an atherogenic lipid profile, atherogenic index was calculated which was found to be significantly high in HFFD fed rats and the risk was reversed in all the treated groups. The decrease in serum cholesterol, LDL cholesterol and triglycerides was in sync with earlier clinical reports (Kumar and Kar, 2017)(Akagawa et al., 2016) wherein supplementation of PQQ (20 mg/day) for 6–12 weeks to 29 healthy adults, ranging from 40 to 57 years with moderate to high cholesterol and triglyceride levels, produced a statistically significant decrease in total cholesterol and LDL cholesterol (Nakano et al., 2015).

Table 4
Effect of PQQ and ATS on fasting serum insulin and insulin sensitivity indices.

	Fasting serum insulin (μIU/mL)	HOMA-IR (Arbitrary values)	QUICKI (Arbitrary values)
Normal control	0.773 ± 0.07	0.181 ± 0.01	0.538 ± 0.010
Positive control (HFFD)	1.627 ± 0.10††††	0.532 ± 0.03††††	0.429 ± 0.006†††
ATS 10	1.328 ± 0.12	0.348 ± 0.04**	0.473 ± 0.016
ATS 20	1.272 ± 0.07	0.279 ± 0.01**	0.473 ± 0.008
PQQ 10	1.004 ± 0.23*	0.303 ± 0.03**	0.483 ± 0.015
PQQ 20	0.701 ± 0.17****	0.236 ± 0.04****	0.515 ± 0.023**
ATS10 + PQQ10	0.933 ± 0.16**	0.208 ± 0.03****	0.526 ± 0.022**
ATS20 + PQQ20	0.732 ± 0.07****	0.200 ± 0.04****	0.536 ± 0.020***

Values are as mean ± SEM (n = 6 for all groups). Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test as a *post-hoc* analysis to identify significant differences among the groups.
HFFD, High fat, 10% fructose diet; ATS 10. Atorvastatin 10 mg/kg; ATS 20. Atorvastatin 20 mg/kg; PQQ 10, Pyrroloquinoline quinone 10 mg/kg; PQQ 20, Pyrroloquinoline quinone 20 mg/kg; ATS 10 + PQQ 10, Atorvastatin + Pyrroloquinoline quinone 10 mg/kg; ATS 20 + PQQ 20, Atorvastatin 20 + Pyrroloquinoline quinone 20 mg/kg.
$^{††††}P < 0.0001$; $^{†††}P < 0.001$ when compared to the control group.
$^{****}P < 0.0001$; $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ when compared with positive control group.

Table 5
Effect of PQQ and ATS on serum lipid profile and atherogenic index.

	Serum Cholesterol (mg/dL)	Serum Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Non-HDL (mg/dL)	Atherogenic index
Normal control	48 ± 4.012	91.2 ± 4.079	30.6 ± 1.43	13.35 ± 1.94	16.8 ± 0.73	48 ± 4.012	0.432 ± 0.06
Positive control (HFFD)	83.5 ± 3.92††††	131.8 ± 6.16††	20.3 ± 1.85†††	60.75 ± 3.30††††	24.35 ± 1.73††††	83.5 ± 3.92††††	3.095 ± 0.29††††
ATS 10	58.2 ± 3.69****	71.8 ± 8.2****	24.6 ± 1	21.85 ± 1.67****	17.08 ± 1.04****	58.2 ± 3.69****	1.362 ± 0.10****
ATS 20	54.4 ± 2.78****	91.8 ± 5.91**	27.4 ± 1.6*	13.4 ± 1.31****	14.92 ± 0.90****	54.4 ± 2.78****	1.014 ± 0.15****
PQQ 10	56 ± 3.536****	87.4 ± 6.56***	27.02 ± 1.33*	22.15 ± 1.45****	15.65 ± 0.82****	56 ± 3.536****	1.098 ± 0.09****
PQQ 20	54.75 ± 3.81****	72.8 ± 8.33****	27.26 ± 1.64*	19.56 ± 2.01****	15.32 ± 0.92****	54.75 ± 3.81****	0.907 ± 0.08****
ATS10 + PQQ10	57.25 ± 2.56****	94.2 ± 5.86**	29 ± 1.73**	19.5 ± 2.08****	17.04 ± 0.64****	57.25 ± 2.56****	1.163 ± 0.12****
ATS20 + PQQ20	54.2 ± 1.46****	84.4 ± 7.87***	31.4 ± 1.28****	14.95 ± 1.72****	13.28 ± 0.67****	54.2 ± 1.46****	0.732 ± 0.053***

Values are as mean ± SEM (n = 6 for all groups). Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test as a *post-hoc* analysis to identify significant differences among the groups.
HFFD, High fat, 10% fructose diet; ATS 10. Atorvastatin 10 mg/kg; ATS 20. Atorvastatin 20 mg/kg; PQQ 10, Pyrroloquinoline quinone 10 mg/kg; PQQ 20, Pyrroloquinoline quinone 20 mg/kg; ATS 10 + PQQ 10, Atorvastatin + Pyrroloquinoline quinone 10 mg/kg; ATS 20 + PQQ 20, Atorvastatin 20 + Pyrroloquinoline quinone 20 mg/kg.
$^{††††}P < 0.0001$; $^{†††}P < 0.001$; $^{††}P < 0.01$ when compared to the control group.
$^{****}P < 0.0001$; $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ when compared with positive control group.

It has been indicated that the alteration of PQQ dietary status in rats modulates mitochondrial, lipid and energy metabolism (Bauerly et al., 2011)(Kumar, 2017). Obese state is associated by decreased anti-inflammatory adiponectin and enhanced pro-inflammatory TNF-α, IL-1β, IL-6and IL-18. Welsh et al., investigated a bidirectional Mendelian randomization strategy to investigate the causal connection between adiposity and inflammation. He found that increased adiposity conferred by adipose tissue and obesity-associated gene and melanocortin receptor 4 single nucleotide polymorphisms resulted in higher C-reactive protein (CRP) levels, with no evidence for any reverse pathway (Welsh et al., 2010). Although this interesting finding requires to be verified and extended to other inflammatory markers, it promotes the emphasis we are giving to adipose tissue in chronic inflammation associated metabolic syndrome (Harris et al., 2013)(Monteiro and Azevedo, 2010). C-reactive protein is traditionally used as nonspecific acute-phase reactant to detect acute infection and inflammation. Elevated concentration of CRP indicates an infection status. This rise in the level of CRP is due to increased cytokine secretion (IL-6) through adipose tissue (Brooks et al., 2010). In correlation with the above statements, biochemical analysis and ELISA quantitation of serum revealed accumulation of total cholesterol and strong induction of inflammatory cytokines by consumption of HFFD. Upon treatment with ATS, PQQ *per se* and together significantly reduced the levels of serum CRP, TNF-α, IL-1β, IL-18, and IL-6 when compared to positive control. PQQ supplementation with ATS decreased the levels of cytokines to a greater extent than individual treatments, which reveals accentuated inhibition of low grade inflammation in the obese rats.

Adipokines is well established to play an significant part in glucose and energy metabolism (Kershaw and Flier, 2004). Obesity, insulin resistance and diabetes are associated with decreased concentrations of adiponectin (Schmidt et al., 2006). Increase in adiponectin was accompanied by anti-inflammatory effects and decreased likelihood of atherosclerosis in healthy non-metabolic subjects (Matsuzawa, 2007). Different studies have shown that the serum concentration of adiponectin is considerably decreased with weight gain and obesity (Ricci and Bevilacqua, 2012)8. In line with these researches, the current research showed that HFFD feeding induces substantial reduction in adiponectin concentration. Both the combinations and *per se* treatment with ATS and PQQ helped in elevating the levels of adiponectin as compared to positive control group. It is assumed that enhanced serum concentrations of inflammatory mediators such as IL-6 and TNF-α that are secreted from adipocytes are accountable for inhibiting and decreasing adiponectin production and secretion (Matsuzawa, 2007).

PQQ a redox cofactor was expected to counter the negative traits of ATS and help in countering the side effects of ATS and improve the metabolic outcomes. To date, our study is the first to investigate the effect of PQQ on chronic low-grade inflammation in HFFD rats and profiled its impact on outcomes of ATS treatment. Possible mechanistic representation pyrroloquinoline quinone attenuation of obesity associated low grade inflammation is shown in Fig. 4. The increase in liver

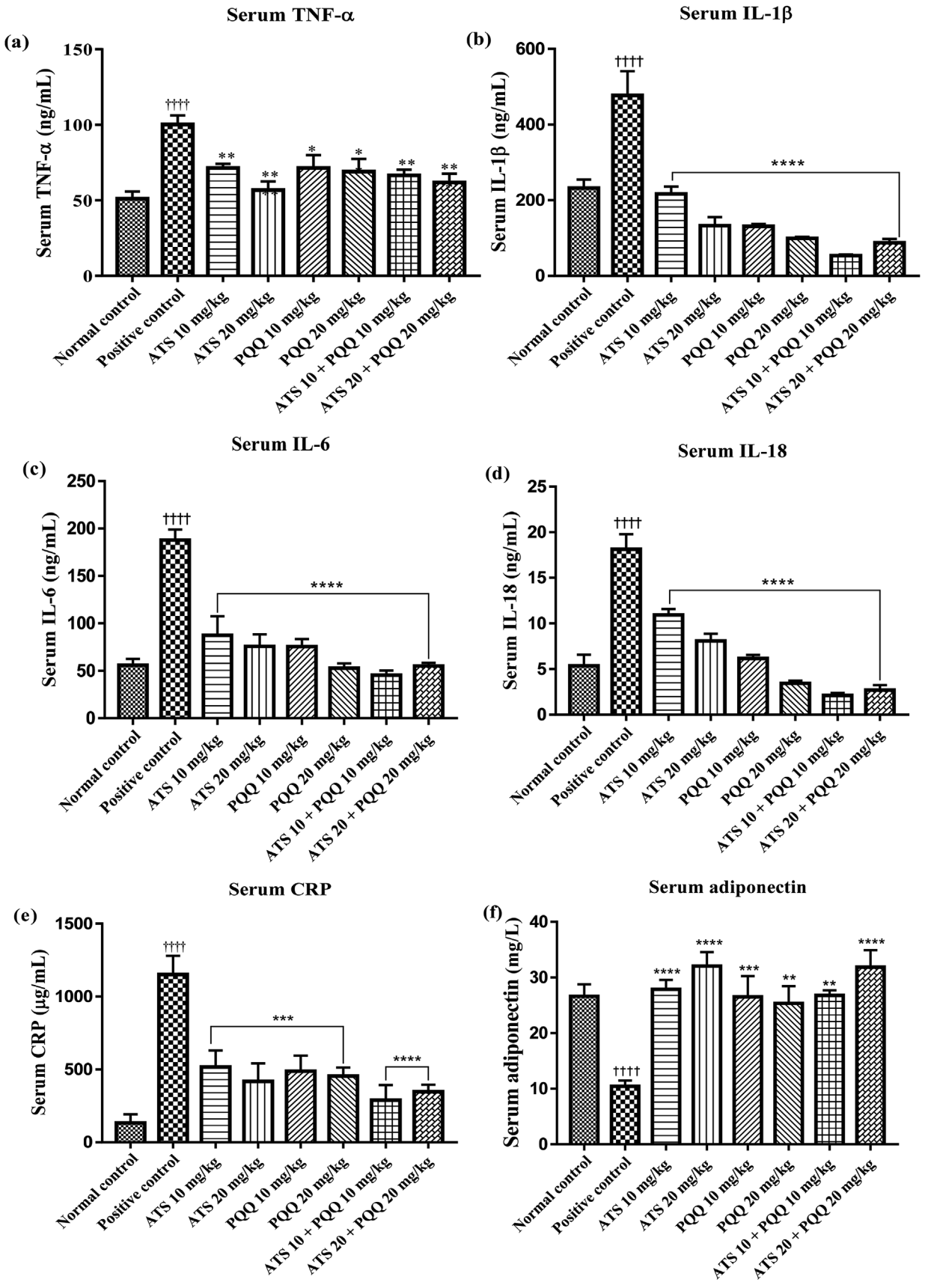


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Fig. 2. Effect of PQQ and ATS on serum a) TNF-α; b) IL-1β; c) IL-6; d) IL-18; e) CRP and f) adiponectin. Values are as mean ± SEM (n = 6 for all groups). Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test as a *post-hoc* analysis to identify significant differences among the groups. HFFD, High fat, 10% fructose diet; ATS 10. Atorvastatin 10 mg/kg; ATS 20. Atorvastatin 20 mg/kg; PQQ 10, Pyrroloquinoline quinone 10 mg/kg; PQQ 20, Pyrroloquinoline quinone 20 mg/kg; ATS 10 + PQQ 10, Atorvastatin + Pyrroloquinoline quinone 10 mg/kg; ATS 20 + PQQ 20, Atorvastatin 20 + Pyrroloquinoline quinone 20 mg/kg. $^{\dagger\dagger\dagger\dagger}P < 0.0001$ when compared to the control group. $^{****}P < 0.0001$; $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ when compared with positive control group.

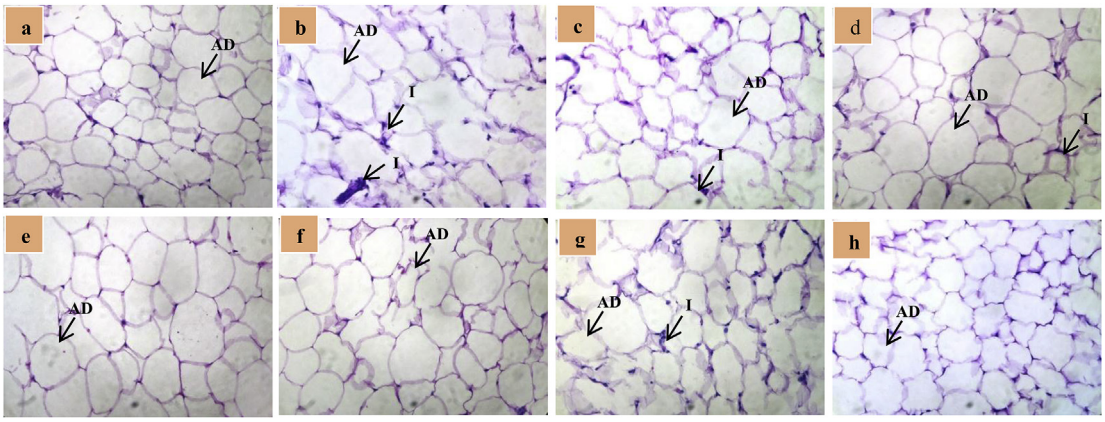

Fig. 3. Effect of PQQ and ATS on histopathological features of intra-abdominal fat in HFFD fed rats. Representative photomicrographs of Haematoxylin and Eosin (H and E) stained sections of adipose tissue of intra-abdominal region showing size and number of adipocytes per high-power field in (a) Normal control; (b) Positive control; (c) ATS (10 mg/kg, *p.o.*); (d) ATS (20 mg/kg, *p.o*); (e) PQQ (10 mg/kg, *p.o.*); (f) PQQ (20 mg/kg, *p.o.*); (g) ATS + PQQ (10 mg/kg, *p.o.*); (h) ATS + PQQ (20 mg/kg, *p.o.*) [H and E × 400]. AD, adipocytes; I, infiltration with inflammatory cells.

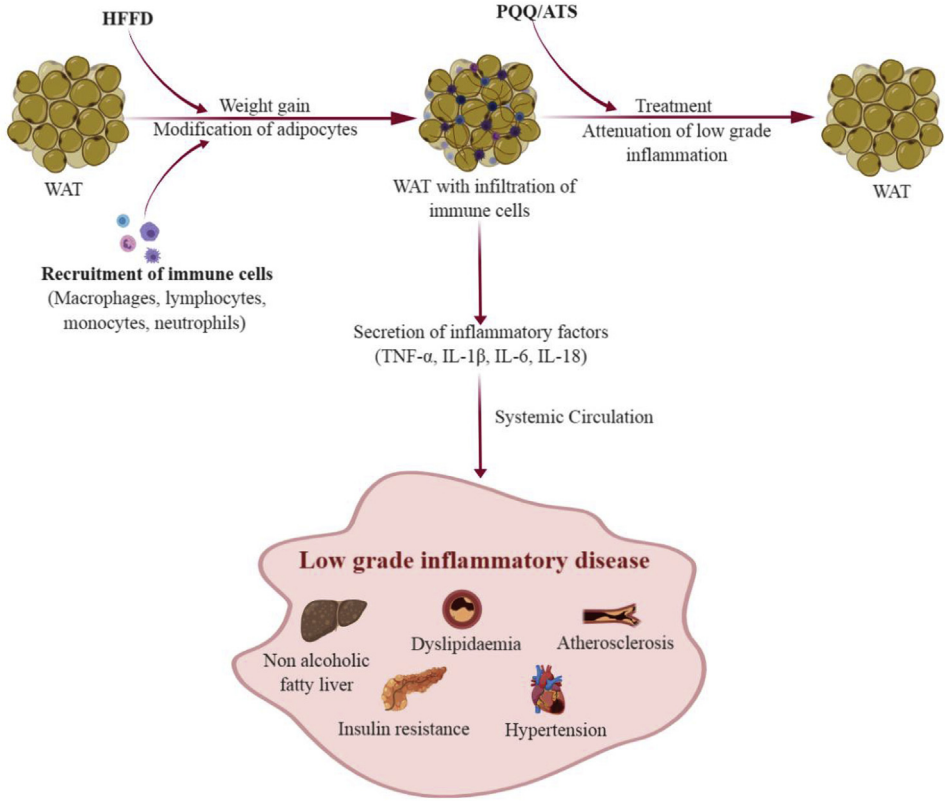

Fig. 4. Possible representation of pyrroloquinoline quinone attenuation of obesity associated low grade inflammation. Abbreviations: High fat, 10% fructose diet (HFFD), White adipose tissue (WAT), Pyrroloquinoline quinone (PQQ), Atorvastatin (ATS), Tumor necrosis factor – alpha (TNF-α), Interleukin-1β ((IL-1β), Interleukin-6 (IL-6), Interleukin-18 (IL-18).

and spleen weight were observed in HFFD fed rats hampering normal development and physiology of these organs. Further, significant increase in intra-abdominal fat (IAF) accumulation was also evident in this group. This undesirable increase in IAF was attributed to the hyperinsulinemia mediated *de-novo* lipogenesis (Sam and Dunaif, 2003). Treatment with ATS, PQQ and its combination significantly abated these changes in organ weights and reduced intra-abdominal fat content.

Histopathological assessment of intra-abdominal fat tissue reveals that consumption of HFFD contributes to enlargement and decline in abdominal adipocytes number. This portrays in increase in BMI and AC/TC ratio and has an obesogenic potential. Both ATS and PQQ *per se* and in combination elicited rise in number of adipocytes, thus reversing the effect of HFFD. These findings were consistent with biochemical

estimations of fat and carbohydrate metabolism status.

5. Conclusion

In conclusion, this investigation provides conclusive evidence regarding benefit of PQQ in treatment of obesity *per se* or in combination with ATS. This preclinical study provides insights on the benefits of PQQ supplementation along with ATS treatment in attenuating low grade inflammation and metabolic derangements in a HFFD rat model.

Authors contribution

Karan Devasani and Anuradha Majumdar conceived and designed the research. Karan Devasani conducted the experiments and wrote the manuscript. Karan Devasani and Anuradha Majumdar analysed and interpreted results.

Compliance with ethical standards

The study was performed according the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India after the approval of Institutional Animal Ethics Committee (IAEC), protocol no. CPCSEA-BCP/2016-02/12.

Conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

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