Available online on 15.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Sitagliptin Recuperates Oxidative Stress and Inflammatory Cytokine Expression in Ovary of PCOS Rats

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is an endocrine, reproductive and metabolic disorder and a major cause of infertility in women. Testosterone propionate (TP) is used to induce PCOS in rats. High calorie diet causes metabolic changes, oxidative stress and PCOS. Sitagliptin (STG) is an inhibitor of dipeptide peptidase (DPP) 4 enzyme used in the treatment of type 2 diabetes. **Objective:** The aim of the study is to investigate the effect of high fat, high fructose diet (HFFD) on TP induced PCOS rats and the role of STG on oxidative stress and inflammation in PCOS. **Materials and methods:** PCOS was induced by administration of TP to normal pellet and HFFD fed rats for 43 days. STG (i.p.) was given for the last 15 days to both groups of rats. Vaginal smear, parameters of oxidative stress, antioxidants and inflammation (TNF-α and IL-6) in ovary were analyzed. **Results:** Vaginal smear from TP rats consisted of persistent leucocytes, a characteristic of PCOS. All the TP administered rats registered significanty elevated levels of glucose, lipids, oxidative stress and inflammatory markers and reduced levels of antioxidants compared to CON rats. STG treatment to PCOS rats reduced hyperglycemia and hyperlipidemia, oxidative stress and inflammation and improved estrus cycle. **Conclusion:** High energy diet aggravated TP-induced changes in oxidative stress and inflammatory cytokines in ovary. STG recuperated the changes induced by TP, suggesting that STG holds potential for PCOS management.

Keywords: PCOS, high fat high fructose diet, sitagliptin, oxidative stress, TNF-α and IL-6

Article Info: Received 12 June 2019; Review Completed 25 July 2019; Accepted 30 July 2019; Available online 15 August 2019

Cite this article as:

Dipti N, Anuradha CV, Sitagliptin Recuperates Oxidative Stress and Inflammatory Cytokine Expression in Ovary of PCOS Rats, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):244-251 **http://dx.doi.org/10.22270/jddt.v9i4-s.3261**

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disease associated with reproductive and metabolic disorders affecting 3.4% women globally.[1] Endocrine disturbances like hyperandrogenism, increased luteinizing hormone (LH) levels, luteinizing hormone:follicular stimulating hormone ratio (LH:FSH ratio) and metabolic changes like hyperinsulinemia, hyperglycemia, insulin resistance and dyslipidemia are observed in PCOS.[2] The phenotypic changes include acne, hirsuitsm, amenorrhea, irregular menstruation and infertility.[3]

Oxidative stress is a pathophysiological condition in which there is loss of balance between free radicals generation and antioxidants levels in living cells. Oxidative stress is considered as one of the potential inducer of PCOS pathogenesis.[4] Oxidative stress in PCOS is found to affect ovarian functions like oocyte maturation, fertilization and embryo development.[5] Studies have evidenced increased oxidative stress markers and reduced antioxidants in human and rat models of PCOS. [6,7]

The treatment and management of PCOS involves both non-pharmacological and pharmacological approaches. Non-pharmacological approaches like diet and exercise, in many PCOS patients are shown to be ineffective.[8] Literature suggests that treatment with various pharmaceutical drugs such as metformin, clomiphene citrate, letrozole, anti-androgen drugs and gonadotropin are useful but have several side effects.[9,10,11] Therefore, it becomes essential to explore alternative drugs for the management of PCOS.

Sitagliptin (STG) is used in the treatment of type 2 diabetes (T2D). The pharmacological action of STG involves the inactivation of dipeptide peptidase 4 enzyme (DPP) 4.[12] DPP 4 removes the N-terminal dipeptide of incretins like glucagon like peptide-1 (GLP-1) which is known to lower blood glucose by increasing the insulin secretion by β-cells. STG by inhibiting DPP 4 increases the bioavailability of GLP-1 causing glucose homeostasis. Besides, glucose lowering effect, antioxidant and anti-inflammatory potential of STG have also been reported.[13,14]

Animal models of PCOS use androgens to induce PCOS. Recently, it is shown that addition of high fat diet to dihydroepiandrostenedione (DHEA) produced exaggerated endocrine and metabolic changes than when DHEA was administered alone.[15] Moreover PCOS subjects are obese due to visceral adiposity. Based on these observations, we hypothesize that high fat, high fructose diet (HFFD) would enhance the changes induced by testosterone propionate (TP) and STG may have beneficial effects in PCOS. The present study was therefore undertaken to analyze the impact of high calorie diet on oxidative stress, antioxidants and inflammatory cytokine expression in TP induced PCOS animals and to explore the recuperative effects of STG in PCOS rats.

MATERIALS AND METHODS

Chemicals, reagents and solvents

TP and crystal violet stain were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. STG was purchased from Merck Pvt. Ltd., USA respectively. Kits for estimation of glucose and insulin were obtained from Agappe Diagnostics Pvt. Ltd., Kerala, India and Accubind, Monobind Inc., CA, USA respectively. Supersensitive polymer-horseradish peroxidase immunohistochemistry detection kit was purchased from Biogenex laboratories, San Ramon, CA, USA. Fine chemicals, solvents and other reagents were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India and Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Diet preparation

HFFD was prepared every day with the following ingredients (g/100 g): fructose 45.0, groundnut oil 10.0, beef tallow 10.0, casein 22.5, DL-methionine 0.3, wheat bran 5.5, vitamin mixture 1.2, and mineral mixture 5.5. The standard rat pellet contained 60% (w/w) starch, 22.08% (w/w) protein and 4.38% (w/w) fat. The energy content of HFFD was 471.25 cal/100 g while that of control diet was 382.61 cal/100 g.

Animals and housing

The study was approved by the Institutional Animal Ethics Committee (IAEC) of Annamalai University (No. 160/1999/CPCSEA/1134) in accordance with the Indian National Law on Animal Care and use. Female albino Wistar rats weighing 50–75 g of age 21 days were used for the study. The animals were procured from Biogene, Laboratory Animal Facility, Bengaluru, India and maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai Nagar. The animals were maintained under standard housing conditions (22-24 °C) in polypropylene cages under 12 h light/dark cycle. The experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design and treatment

The animals were allowed to acclimatize for a week, after which the animals were randomly distributed into five groups each comprising of six animals (n = 6) and were maintained for 43 days.

Group 1: Animals received normal pellet and served as control group

Group 2: Animals received normal pellet and TP for 43 days

Group 3: Animals received HFFD and TP for 43 days

Group 4: Animals received normal pellet, TP for 43 days and STG for the last 15 days

Group 5: Animals received HFFD and TP for 43 days and STG for the last 15 days.

The animals had free access to diet and water. TP was administered at the dose of 1 mg/100 g bw/day in olive oil, i.p. and STG at the dose of 30 mg/kg bw/day in 0.9 % saline by intragastric intubation. The dosage used is based on previous reports.[16,17] Body weight of the animals was measured the beginning and at the end of the study. Estrus cycle of rats was monitored by microscopic examination of the vaginal smear which was collected early morning from 8th day till 43rd day. At the end of the 43 days, rats were fasted overnight, anaesthetized with ketamine hydrochloride (30 mg/kg, i.p.) and killed by cervical dislocation.

Vaginal smear preparation

The vaginal smear was obtained using aseptic moist (in 0.9% saline) cotton swab rolled delicately around the vaginal opening and was transferred onto a glass slide. The slides were allowed to dry at room temperature and stained with crystal violet followed by gentle wash to remove the excess stain. The stained slides were dried, fixed using glycerol and covered with cover slip.[18] The image was taken under Olympus CX 4 microscope [40X], (Olympus Corporation, Tokyo, Japan).

Sampling of blood and tissue

Blood was collected from the retino-orbital plexus in tubes containing the anticoagulant EDTA for separation of plasma by centrifugation (1500×g, 15 min). Red blood cells were further processed to prepare hemolysate for the assay of enzymatic and non-enzymatic antioxidants. Ovary tissue was dissected and washed immediately in ice-cold saline (0.9% sodium chloride). Ovary homogenate (10%) was prepared in ice-cold 0.1 M Tris-HCl buffer, pH 7.4. Portions from ovary tissue were placed in 10% formalin for performing immunohistochemical studies.

Estimation of biochemical parameters

The levels of glucose and insulin in plasma were determined using kits. The levels of total cholesterol (TC) and triglyceride (TG) in plasma and ovary were measured by the methods of Zlatkis *et al.* (1953) [19] and Foster *et al.* (1973).[20]

Evaluation of oxidative stress

Thiobarbituric acid reactive substances (TBARS) in plasma and ovary homogenate was measured by the method of Niehaus and Samuelsson (1968).[21] Lipid hydroperoxides (LHP) levels were quantitated by the method of Jiang *et al.* (1992).[22] The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and the level of glutathione (GSH) were assayed in hemolysate and ovary homogenate by methods described elsewhere.[23]

Analysis of inflammatory markers

The cytokines TNF-α and IL-6 were assayed using immunohistochemistry. For this, the ovary sections was deparaffinized by baking for 1 h at 60°C followed by dewaxing with xylene. Sections were then rehydrated with graded concentrations of isopropyl alcohol and subjected to antigen retrieval for 3 min using citrate buffer (0.1M, pH 6.0). Antigen retrieved sections were incubated in peroxide blocking reagent for 10 min and then rinsed with phosphate buffer followed by incubation for 10 min with power block solution. Nonspecific binding was minimized by washing the sections with 3% BSA in phosphate-buffered saline for 30 min. Sections were incubated overnight with the primary antibodies. Different dilutions of primary antibodies were used: TNF-α (1:100) and IL-6 (1:100). The sections were rinsed with phosphate buffer and incubated with super enhancer reagent for 30 min. The rinsed sections were incubated with Polymer-HRP reagent. The sections were washed with buffer and incubated with 3, 3′-diaminobenizidine (DAB) substrate solution for 5 min and counterstained with Mayer's hematoxylin. The sections were photographed under the Olympus CX 4 microscope [40X], (Olympus Corporation, Tokyo, Japan).

Statistical analysis

SPSS software, version 20.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Significant difference in means between the groups was analyzed using one-way ANOVA followed by Tukey's test for multiple comparisons. Two-way ANOVA was used to evaluate the interactive effect of HFFD and STG treatment on oxidative stress markers and antioxidants. Values are presented as means ± SD (n = 6). A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Confirmation of PCOS

Induction of PCOS was confirmed by examining the vaginal smear cytology. The distinct cell types associated during different stages of estrus cycle are epithelial cells with few keratinocytes in the proestrus stage; keratinocytes in the estrus; keratinocytes, leukocytes and epithelial cells in the metestrus; predominanlty leucocytes in the diestrus.

Figs. 1a-h show the representative photographs of vaginal smears obtained from the different experimental groups. Figs. 1a-d represent vaginal smear from CON group. The presence of nucleated epithelial cells with few keratinocytes are seen in Fig. 1a signifying proestrus stage. Fig. 1b shows the presence of keratinocytes signifying estrus stage. Three distict cell types namely keratinocytes, leukocytes and epithelial cells are found in Fig. 1c indicating metestrus stage. Presence of leucocytes in Fig. 1d indicates diestrus stage. Dominance of leucocytes was noted in CD+TP group and HFFD+TP group (Figs. 1e and 1f respectively). The leucocytes were denser in HFFD+TP rats (Fig. 1f) than CD+TP rats (Fig. 1e). STG treated TP groups display mainly epithelial cells and very few leukocytes indicating the recovery of PCOS rats from diestrus to proestrus stage (Figs. 1g and 1h).

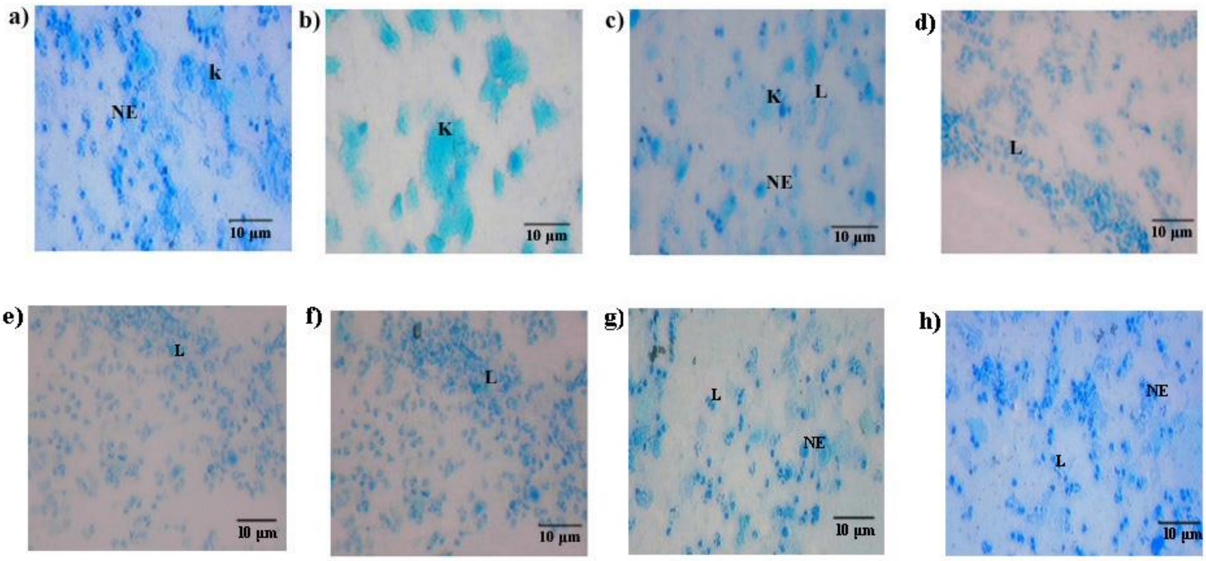

Fig. 1. Representative photographs of different cell types of vaginal smears obtained from experimental rats during estrus cycle. a) Appearance of nucleated epithelial cells and keratinocytes in proestrus stage from control rats. **b)** Presence of keratinocytes in estrus stage from control rats. **c)** Presence of keratinocytes, leukocytes and epithelial cells in metestrus stage from control rats. **d)** Occurrence of leucocytes in diestrus stage from control rats. **e)** Occurrence of persistent leucocytes from CD+TP rats. **f)** Existence of persistent leucocytes from HFFD+TP rats. **g)** Appearance of epithelial cells and few leukocytes from CD+TP+STG treated rats. **h)** Appearance of epithelial cells and leukocytes from HFFD+TP+STG treated rats. K-keratinocytes; NE-nucleated epithelial cells; L-leukocytes. [magnification 40X]. Scale bar, 10 µm.

Body weight

Table 1 shows the initial and final body weights of the animals. The initial body weight of the animals was between 50-52 g. A significant gain in final body weight of rats was observed in all the groups. TP administered rats maintained on HFFD gained more body weight than those given normal pellet. STG+TP treated rats maintained on HFFD (Group 5) or normal diet (Group 4) showed decrease in body weight compared to respective STG untreated TP administered rats (Group 2 and 3 respectively). Diet and treatment showed independent as well as interactive effect on final body weight (Table 1).

Glucose and insulin

The levels of glucose and insulin from plasma are provided in Table 1. Elevated levels of glucose and insulin were observed in all the TP given animals compared to CON animals. Significant rise in the levels of both glucose and insulin were noted in TP animals placed on HFFD diet (Group 3) compared to TP animals given normal diet (Group 2). However, STG treated groups on either diet showed restoration of the levels compared STG untreated groups. An independent effect of diet and treatment was seen for both glucose and insulin levels but showed no interactive effect (Table 1).

Table 1. Initial and final body weight, glucose and insulin levels of experimental rats

Groups	CON	CD+TP	HFFD+TP	CD+TP+STG	HFFD+TP+STG	TWO-WAY ANOVA DIET	STG Treatment	INTER
Body weight								
Initial body weight (g)	50.11±0.71	51.41±1.06	51.27±0.88	50.99±0.45	51.49±0.85	$p <$ 0.00	NS	NS
Final body weight (g)	67.67±79.27[a]	79.27±0.55[b]	91.91±0.86[c]	71.40±0.81[d]	86.24±1.21[e]	$p <$ 0.00	$p < 0.00$	$p <$ 0.00
Glucose and insulin								
Glucose (mg/dL)	89.38±3.89[a]	136.44±2.29[b]	164.09±1.02[c]	121.80±4.15[d]	152.38±0.73[e]	$p < 0.00$	$p < 0.00$	NS
Insulin (μU/mL)	15.09±0.80[a]	25.40± 0.98[b]	36.12±0.15[c]	18.57±0.76[d]	28.51±1.13[e]	$p < 0.00$	$p < 0.00$	NS

CON- normal pellet fed rats; CD+TP- normal pellet fed rats+TP; HFFD+TP- high fat, high fructose diet fed rats+TP; CD+TP+STG- normal pellet fed rats+TP+STG treated; HFFD+TP+STG- HFFD fed rats+TP+STG treated; INTER-Interaction; NS-not significant. Values are means ± SD of rats. Values sharing different superscripts are significantly different from one another. One-way ANOVA was used to determine the significance of means between groups. $p < 0.05$ was considered significant. Two-way ANOVA was used to evaluate the effect of HFFD and STG treatment between the groups. $p < 0.05$ was considered significant.

Lipids

Table 2 shows TC and TG levels in ovary which were found to be significantly elevated in all TP administered animals compared to CON animals. The increase was more in HFFD fed TP rats compared to TP rats given normal pellet. Treatment of HFFD+TP and CD+TP groups with STG significantly decreased the levels respectively but not upto the control level. Diet and treatment independently affected TC and TG levels but interactive effect was seen only TG (Table 2).

Table 2. TC and TG levels in ovary of experimental rats

Groups	CON	CD+TP	HFFD+TP	CD+TP+STG	HFFD+TP+STG	Two-Way ANOVA DIET	STG Treatment	INTER
Plasma								
TC (mmol/L)	2.40±0.02[a]	3.42±0.02[b]	5.42±0.01[c]	2.85±0.02[d]	4.86±0.03[e]	$P < 0.00$	$P < 0.00$	$P < 0.52$
TG (mmol/L)	1.43±0.01[a]	2.33±0.01[b]	3.43±0.02[c]	1.94±0.01[d]	2.89±0.01[e]	$P < 0.00$	$P < 0.00$	$P < 0.00$
Ovary								
TC (mg/g tissue)	2.52±0.02[a]	3.77±0.01[b]	5.91±0.01[c]	3.00±0.02[d]	5.35±0.01[e]	$P < 0.00$	$P < 0.00$	$P < 0.00$
TG (mg/g tissue)	1.55±0.01[a]	2.52±0.01[b]	3.67±0.03[c]	2.10±0.01[d]	3.22±0.01[e]	$P < 0.00$	$P < 0.00$	$P < 0.11$

CON- normal pellet fed rats; CD+TP- normal pellet fed rats+TP; HFFD+TP- high fat, high fructose diet fed rats+TP; CD+TP+STG- normal pellet fed rats+TP+STG treated; HFFD+TP+STG- HFFD fed rats+TP+STG treated; INTER-Interaction; NS-not significant. Values are means ± SD of rats. Values sharing different superscripts are significantly different from one another. One-way ANOVA was used to determine the significance of means between groups. $p < 0.05$ was considered significant. Two-way ANOVA was used to evaluate the effect of HFFD and STG treatment between the groups. $p < 0.05$ was considered significant.

Oxidative stress markers

Table 3 illustrates the oxidative stress markers from plasma and ovary of experimental rats. TP administered animals exhibited significantly elevated levels of TBARS and LHP compared to CON rats. The levels were significantly elevated in TP rats maintained on HFFD than those fed normal diet. Significant reduction in levels the oxidative stress markers was observed in plasma and ovary of STG treated HFFD+TP and CD+TP rats compared to groups deprived of STG treatment. Two-way ANOVA result showed significant independent as well as interactive effects of diet and treatment on oxidative stress (Table 3).

Table 3. Levels of TBARS and LHP in experimental rats

Groups	CON	CD+TP	HFFD+TP	CD+TP+STG	HFFD+TP+STG	Two-Way ANOVA		
						DIET	STG Treatment	INTER
Plasma								
TBARS (μmol/dL)	1.20±0.02[a]	4.27±0.05[b]	4.94±0.02[c]	2.88±0.02[d]	3.43±0.02[e]	$p < 0.00$	$p < 0.00$	$p < 0.001$
LHP (nmol/dL)	1.75±0.02[a]	4.71±0.02[b]	5.73±0.02[c]	3.63±0.01[d]	4.48±0.02[e]	$p < 0.00$	$p < 0.00$	$p < 0.00$
Ovary								
TBARS (nmol/mg protein)	1.23±0.02[a]	5.28±0.01[b]	6.34±0.01[c]	3.40±0.02[d]	4.65±0.02[e]	$p < 0.00$	$p < 0.00$	$p < 0.00$
LHP (nmol/mg protein)	2.09±0.01[a]	5.54 ±0.02[b]	7.50±0.02[c]	3.96±0.01[d]	4.95±0.01[e]	$p < 0.00$	$p < 0.00$	$p < 0.001$

CON- normal pellet fed rats; CD+TP- normal pellet fed rats+TP; HFFD+TP- high fat, high fructose diet fed rats+TP; CD+TP+STG- normal pellet fed rats+TP+STG treated; HFFD+TP+STG- HFFD fed rats+TP+STG treated; INTER-Interaction; NS-not significant. Values are means ± SD of rats. Values sharing different superscripts are significantly different from one another. One-way ANOVA was used to determine the significance of means between groups. $p < 0.05$ was considered significant. Two-way ANOVA was used to evaluate the effect of HFFD and STG treatment between the groups. $p < 0.05$ was considered significant.

Antioxidants status

Tables 4 and 5 illustrate the antioxidant status (enzymatic and non-enzymatic) in the hemolysate and ovary respectively of experimental rats. TP treated animals exhibited significantly reduced activities of SOD, CAT, GPx and GSH in both hemolysate and ovary as compared to CON rats signifying the presence of ROS-antioxidant imbalance. Between the diet groups the levels were significantly decreased in HFFD+TP rats compared to CD+TP rats. STG supplementation in both the diet groups improved the levels significantly in hemolysate (Table 4) as well as in ovary (Table 5) compared to respective STG untreated groups. The result of two-way ANOVA showed independent influence of diet and treatment on both enzymatic and non-enzymatic antioxidants levels. No interactive effect was seen for levels of CAT hemolysate and ovary tissue, GPx in hemolysate, and GSH in ovary.

Table 4. Antioxidants levels in hemolysate of experimental rats

Groups	CON	CD+TP	HFFD+TP	CD+TP+STG	HFFD+TP+STG	Two-Way ANOVA		
						DIET	STG Treatment	INTER
SOD (U/mg Hb)	3.89±0.37[a]	2.37±0.01[b]	1.27±0.05[c]	2.75±0.20[d]	1.99±0.07[e]	$P < 0.00$	$P < 0.00$	$P < 0.04$
CAT (μmoles of H_2O_2 consumed/min/ mg Hb)	44.77±1.53[a]	32.95±0.65[b]	19.66±0.86[c]	41.44±0.72[d]	28.78±0.70[e]	$P < 0.00$	$P < 0.00$	$P < 0.47$
GPx (μmoles GSH consumed/min/ mg Hb)	8.62 ±0.69[a]	5.57±0.19[b]	3.93±0.47[c]	6.52±0.28[d]	4.71±0.07[e]	$P < 0.00$	$P < 0.00$	$P < 0.61$
GSH (mg/dL plasma)	21.04±0.59[a]	15.38±0.34[b]	8.20±0.50[c]	17.44±0.26[d]	12.31±0.34[e]	$P < 0.00$	$P < 0.00$	$P < 0.00$

CON- normal pellet fed rats; CD+TP- normal pellet fed rats+TP; HFFD+TP- high fat, high fructose diet fed rats+TP; CD+TP+STG- normal pellet fed rats+TP+STG treated; HFFD+TP+STG- HFFD fed rats+TP+STG treated; INTER-Interaction; NS-not significant. Values are means ± SD of rats. Values sharing different superscripts are significantly different from one another. One-way ANOVA was used to determine the significance of means between groups. $p < 0.05$ was considered significant. Two-way ANOVA was used to evaluate the effect of HFFD and STG treatment between the groups. $p < 0.05$ was considered significant. U = enzyme concentration required to produce 50% inhibition of chromogen formation in one minute under standard condition.

Table 5. Antioxidants levels in ovary of experimental rats

Groups	CON	CD+TP	HFFD+TP	CD+TP+STG	HFFD+TP+STG	Two-Way ANOVA Treatment		
						DIET	STG	INTER
SOD (U/mg protein)	4.01±0.13[a]	3.07±0.14[b]	2.06±0.11[c]	3.84±0.11[d]	2.59±0.12[e]	$p < 0.00$	$p < 0.00$	$p < 0.03$
CAT (µmoles of H_2O_2 consumed/min/mg protein)	45.59±0.49[a]	26.63±0.67[b]	14.05±0.62[c]	35.93±0.56[d]	23.58±0.42[e]	$p < 0.00$	$p < 0.00$	$p < 0.64$
GPx (µmoles GSH consumed/min/mg protein)	6.80±0.07[a]	4.79±0.09[b]	2.43±0.06[c]	5.48±0.16[d]	3.29±0.05[e]	$p < 0.00$	$p < 0.00$	$p < 0.04$
GSH (µg/mg plasma)	44.24±0.83[a]	28.16±0.51[b]	17.96±0.67[c]	34.23±0.92[d]	24.90±0.19[e]	$p < 0.00$	$p < 0.00$	$p < 0.12$

CON- normal pellet fed rats; CD+TP- normal pellet fed rats+TP; HFFD+TP- high fat, high fructose diet fed rats+TP; CD+TP+STG- normal pellet fed rats+TP+STG treated; HFFD+TP+STG- HFFD fed rats+TP+STG treated; INTER-Interaction; NS-not significant. Values are means ± SD of rats. Values sharing different superscripts are significantly different from one another. One-way ANOVA was used to determine the significance of means between groups. $p < 0.05$ was considered significant. Two-way ANOVA was used to evaluate the effect of HFFD and STG treatment between the groups. $p < 0.05$ was considered significant. U = enzyme concentration required to produce 50% inhibition of chromogen formation in one minute under standard condition.

Immunohistochemical localization of TNF-α and IL-6 in ovary

Fig. 2 represents the immunohistochemical localization of TNF-α (Figs. 2a-e) and IL-6 (Figs. 2f-j) in ovary of experimental rats. The expression of TNF-α in ovary was more intense in TP treated rats on HFFD (Fig. 2c) as compared to rats on normal diet (Fig. 2b). A marked reduction in immunoreactivity of TNF-α was observed in STG supplemented groups (Figs. 2d and 2e) as compared to STG unsupplemented groups (Figs. 2b and 2c). CON rats displayed very mild immunoreactivity (Fig. 2a).

Similarly, increased immunoreactivity for IL-6 in ovary tissue was noted in TP administered rats fed with HFFD (Fig. 2h) as compared to TP rats fed normal pellet (Fig. 2g). The intensity of the colour was found to be reduced in STG treated groups (Figs. 2i and 2j) compared to STG untreated groups (Figs. 2g and 2h). The intensity of immunoreactivity was very less in CON group (Fig. 2f)

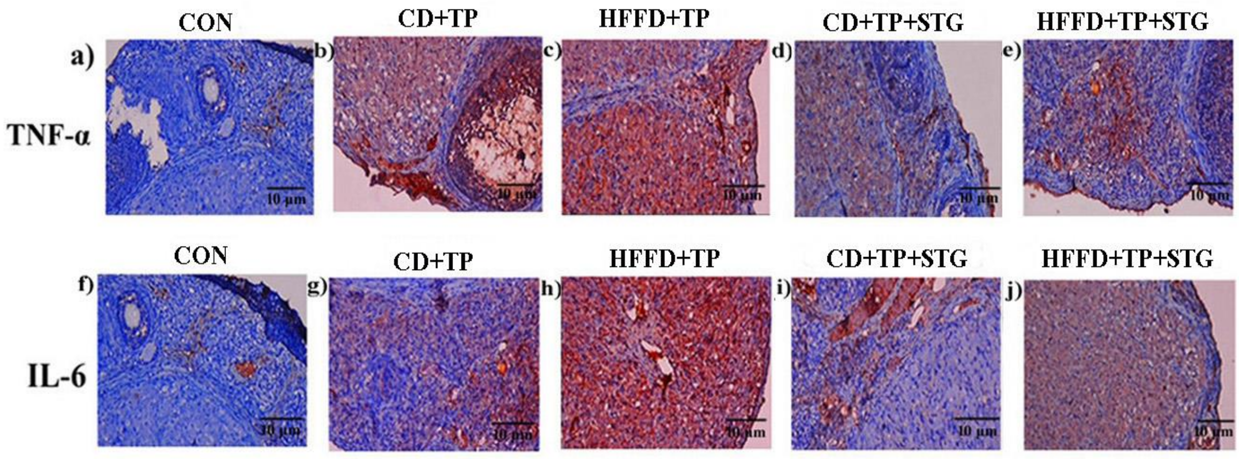

Fig. 2 Representative photomicrographs of immunohistochemical localization of TNF-α and IL-6 in rat ovary. a-e) TNF-α and **f-j)** IL-6. CON- normal pellet fed rats; CD+TP- normal pellet fed rats+TP; HFFD+TP- high fat, high fructose diet fed rats+TP; CD+TP+STG- normal pellet fed rats+TP+STG treated; HFFD+TP+STG- HFFD fed rats+TP+STG treated. The presence of antigen–antibody interaction displayed brown colour and the absence of antigen–antibody interaction displayed counterstained haematoxylin colour that appeared blue colour [magnification 40X]. Scale bar-10 µm

DISCUSSION

The study demonstrated two major findings i) administration of high calorie diet exaggerates TP induced oxidative stress and inflammation and ii) STG alleviates oxidative stress and inflammation in ovary of PCOS rats.

The disruption of the delicate balance between oxidants and antioxidant system resulting in oxidative stress has been reported by several investigators in PCOS models.[24,25] Oxidative stress plays a central role in the progression of female infertility and has been shown to affect a variety of physiological functions such as folliculogenesis, oocyte maturation, ovarian steroidogenesis, ovulation, fertilization, implantation, formation of blastocyst, luteolysis and luteal maintenance in pregnancy.[4]

A study by Nasiri *et al.* (2015) [26] reported the influence of abdominal obesity on oxidative stress in women. Elevated levels of lipid peroxides and reduced level of total antioxidant capacity were observed in both PCOS and normal women having abdominal obesity as compared to normal and PCOS women without abdominal obesity. This study suggests that fat accumulation plays a contributory role in progression of oxidative stress.[26]

According to Keane *et al*. (2015) oxidative stress is mainly driven by hyperglycaemia and hyperlipidemia.[27] In this study, TP rats fed HFFD or normal diet displayed both hyperglycaemia and hyperlipidemia resulting in oxidative stress. Previous findings have registered that HFFD feeding to animals cause decreased activity enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH) antioxidants and increased activity of oxidative stress markers.[28,29] In this study, HFFD feeding in TP rats resulted in elevated levels of oxidative stress markers and decreased levels of enzymatic and non-enzymatic antioxidants as compared to normal diet fed TP rats which can be attributed to hormonal changes induced by TP and metabolic changes induced by HFFD.

TNF-α and IL-6 are capable of regulating the reproductive events such as folliculogenesis, ovulation and fertilization.[30] Oxidative stress and inflammation are proposed to be interlinked. ROS generated by oxidative stress act as intracellular messengers to induce the synthesis of cytokines via activating the nuclear transcription factor (NF-κB-p65) gene which upregulates the expression of cytokines.[31] Activation of NF-κB and an increase in TNF-α have been described in in PCOS women.[32] The expression of TNF-α and IL-6 were increased in all TP administered rats compared to control rats and expression was more in TP rats on HFFD than those on CD.

STG restored the levels of enzymatic, non-enzymatic antioxidants and oxidative stress status. Thus, STG by virtue of its antioxidant and free radical scavenging activities [29,33] is capable of modulating antioxidant enzyme activities and suppressing the oxidative stress markers in PCOS rats. The restoration of oxidative stress by STG might be due its glucose and lipid lowering potential, which was decreased in STG treated groups in our study. Studies demonstrated that STG treatment in mice fed high fat diet (HFD) lowered the levels of TNF-α and IL-6. [34] Normalization of TNF-α and IL-6 in TP rats following STG treatment signifies that apart from its antioxidant and radical scavenging activities, STG has anti-inflammatory effects that might have blocked or prevented the expression of inflammatory cytokines (TNF-α and IL-6).

CONCLUSION

The results obtained in this study show that inclusion of high energy diet promotes PCOS pathogenesis by aggravating oxidative stress and inflammatory cytokine expression. STG treatment recovered the levels of antioxidants and reduced oxidative stress and inflammation. Therefore, it may be concluded that gliptins may offer a therapeutic potential in management of oxidative stress in PCOS subjects.

ACKNOWLEDGEMENTS

The authors thank DST-FIST and UGC-SAP support for the facilities developed in the Department of Biochemistry and Biotechnology, Annamalai University for executing the present study. One of the authors Ms. Dipti Nanda is a recipient of Research Fellowship in Sciences for Meritorious Students (RFSMS) (F-25-1/2014-2015(BSR/7-10/2007) awarded by University Grants Commission (UGC), New Delhi, India.

CONFLICT OF INTEREST

None

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