The Egyptian Journal of Hospital Medicine (January 2024) Vol. 94, Page 621-629

The sodium glucose transporter 2 inhibitor Dapagliflozin Regulates kisspeptin and GABA receptors mRNA expression in hypothalamic arcuate nucleus in polycystic ovary rat model , Can it be a therapeutic target ?

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a reproductive disease that causes metabolic, endocrine and cardiovascular effects. Dapagliflozin (DAPA) is a sodium glucose transporter 2 (SGLT-2) inhibitors that control glucose level, and improve insulin sensitivity, DAPA improved sex hormones profile and ovulation rate in obese mouse model.

Objective: The study aimed to detect if there is a role of DAPA on ovarian function in estradiol-induced PCOS rats.

Material and methods: Thirty rats were divided into 2 groups. Group I (control) were given 0.5 ml saline intramuscular (IM) once, after 60 days, they were given. 0.9% saline by gastric gavage. Group II (PCOS-induced group) where rats were administered 4 mg/kg of estradiol valerate by single IM injection, 60 days later. PCO group was subdivided into subgroup IIa where rats were given 0.9% saline. Subgroup IIb included rats that were given DAPA 5 mg/kg/day. Both subgroups were administered by gastric gavage for 4 weeks. At end of experiment, serum sex hormones profile, insulin, glucose, ovarian oxidative stress and inflammatory markers, ovarian histopathology, transforming growth factor B1 (TGF-B1) immunohistochemistry, hypothalamic kisspeptin and GABA B receptor gene expression were estimated.

Results: In PCO group IIa, value of kisspeptin expression was increased significantly ($p < 0.01$), value of GABA B receptor expression was decreased significantly ($P < 0.01$), compared to Dapagliflozin-treated PCO group IIb. The same opposing results when both compared to normal control group I ($P< 0.001$). Moreover, there was a significant increase in kisspeptin expression ($P<0.05$) and significant decrease in GABA B receptor expression ($P< 0.01$) in Dapagliflozin-treated PCO group IIa compared to normal control group I.

Conclusion: DAPA improved inflammatory status, oxidative stress markers, hormonal profile, ovarian histological structure and decreased TGF-B1 immunoreactivity and kisspeptin and increased GABA B receptor gene expression. DAPA can be used as a therapeutic target for PCOS.

Keywords: Polycystic ovary syndrome, Estradiol, Dapagliflozin, Glucose transporter 2 inhibitors.

INTRODUCTION

PCOS is characterized by ovarian polycystic changes, it can cause female infertility, menstrual irregularity, hyperandrogenism and ovulatory disturbance, which is caused by increased ovarian granulosa cells (GC) apoptosis [1].

In addition, disturbance in pulsatile manner of gonadotrophin releasing hormone (GnRH) release under the effect of hypothalamic kisspeptin, which is considered the master regulator of GnRH neurons. In addition to metabolic disorders involving insulin resistance (IR), chronic inflammation and oxidative stress [1, 2]. SGLT-2 inhibitors are drugs that lower blood glucose levels by preventing glucose reabsorption in renal proximal tubules [3].

DAPA is highly selective SGLT-2i that give glycaemic control, weight loss, improved insulin sensitivity, reduced oxidative stress and inflammation. It was the first SGLT-2 inhibitors to be approved for managing diabetes mellitus type 2 [3, 4].

In addition, DAPA improved sex hormones profile and ovulation rate in obese mouse model [5]. SGLT-2 inhibitors may be effective for many characters related to PCOS, including insulin resistance (IR), oxidative stress, and inflammation [6].

This study aimed to assess if DAPA has a therapeutic role in polycystic ovary syndrome.

MATERIALS AND METHODS

Experimental animals: 30 adolescent virgin female albino rats of local strain weighing 180-200 gram were obtained from Veterinary Medicine Faculty, Zagazig University. The animals were housed in steel wire cages. They received food and water ad libitum and were left for two weeks for acclimatization before start of the experiment.

Methods:

Grouping of animals: Group I (control) (n = 10 rats)**:** Rats were given 0.5 ml saline IM once. Then after 60 days, they were given .0.9% saline by gastric gavage every day for 4 weeks.

Group II (PCOS-induced group) (n= 20 rats): Rats were administered 4 mg/kg of estradiol valerate by single IM injection [7], 60 days later, PCO group was subdivided into two subgroups (n = 10 rats/group).

Subgroup II a: rats were given 0.9% saline by gastric gavage every day for 4 weeks. **Subgroup II b:** rats were given DAPA (5 mg/kg/day) by gastric gavage every day for 4 weeks [8].

Induction of PCOS: Single dose of estradiol valerate (EV) causes irregularity in reproductive cycles with

Received: 02/10/2023
Accepted: 02/12/2023

anovulation and polycystic ovaries. The ovarian changes are similar to PCOS women [7].

Determination of Sexual cycle: Vaginal smears were obtained daily by washing of vagina by saline, then examined by microscope during the treatment period, regular cycles' duration is 4 or 5 days [9].

Proestrus phase; many live epithelium, the margins are smooth, **estrus phase**; large cornified cells margins are irregular, **metestrus phase**; several cornified cells and leukocytes infiltration, **diestrus** phase; small leukocytes without cornified cells. Persistent estrus is presence of cornified cells for at least 10 continuous days, this indicates development of follicular cysts.

Sampling of blood:

One day following the last gastric gavage of DAPA, blood samples were obtained from orbital sinus, then allowed to clot, and centrifuged at 3000 rpm for 15 minutes, and was stored frozen at -20 ºC

Biochemical analysis:

Commercial rat kits were used for assaying: -Serum LH, FSH, estradiol, progesterone and testosterone by (ELISA), glucose and insulin (BioSource Europe S.A.- Rue de l'Industrie, 8-B- 1400 Nivelles-Belgium). Homeostasis model assessment (HOMA) was calculated, HOMA-IR = insulin (μIU/mL) X glucose (mg/dl)/405.

Measurements of ovarian inflammatory cytokines and oxidative stress markers: Ovarian tissues were dissected, and homogenized, then centrifuged 5000 rpm for 5 min, supernatant was used for ELISA to estimate the levels of TNF-α, IL-6, SOD and MDA [10].

Real-time polymerase chain reaction (RT-PCR) for gene expression of Kisspeptin and GABA amino butyric acid (GABA) B receptors in hypothalamic arcuate nucleus [11]:

One day following the last gastric gavage, rats were subjected to anesthesia by ketamine and xylazine injection. Samples from hypothalamic arcuate nucleus were dissected and stored at -80 °C. Total RNA was extracted and was reverse-transcribed into cDNA. We used internal control genes GAPDH and β-actin primers, according to manufacturer instructions.

Kiss1	
Forward primer,	TGCTGCTTCTCCTCTGTG
Reverse primer,	CCAGGCATTAACGAGTTCC
Gabbr1	
Forward primer,	CACGAAGAAGGAGGAGAAG
Reverse primer,	CAGATGGCAAGAGTCAGG
Gapdh	
Forward primer,	TCAACGGCACAGTCAAGG
Reverse primer,	CTCAGCACCAGCATCACC
B-actin	
Forward primer,	CGTGACATCAAGGAGAAG
Reverse primer,	GAAGGAAGGCTGGAAGAG

Ovarian histopathology and immune-histochemistry: According to **Suvarna *et al.* [12]:** (1) Fixation: in formalin for 48 hours. (2) Embedding in paraffin wax. (3) 5 μm thick paraffin sections were obtained. (4) Staining: a-Histological stains: by Hematoxylin and Eosin, and b-Immunohistochemical study where the anti-TGF-β antibody was used for detection of TGF-β according to manufacturer instructions.

Morphometric study according to Mohammadghasemi *et al.* [13]: Sections of Hematoxylin and Eosin were analyzed morphometrically by image analyzer computer system. **3 intensity score:** (S1) for weak intensity, (S2) for moderate intensity (S3) for strong intensity. In addition, the area of TGFB1 positive reaction was measured in TGFB1 immune stained sections of ovary under 400 high power fields.

Ethical approval: The study was approved by Ethics Committee of Faculty of Medicine, Zagazig University (ZU-ICUC/3/F/442/2023). Rats were handled according to National Institutes of Health (NIH) guidelines for animal experimentation.

Statistical Analysis

SPSS version 22 for Windows® was used to code, process, and analyse the gathered data. To determine how two or more sets of qualitative variables differ from one another, the Chi square test (χ^2) was used. The statistical information was presented as mean ± SD and one-way ANOVA analysis was used. The normally distributed variables (parametric data) in two independent groups were compared using the independent samples t-test. When it was equal to or less than 0.05, the p-value was deemed significant.

RESULTS

Table (1) statistical analysis of serum levels of LH levels (μIU/ml), FSH (μIU/ml), estradiol (pg/ML), free testosterone (pg/ML), and progesterone (ng/ml) among the studied groups. The value of LH level, estradiol level and free testosterone were increased significantly in PCO group IIa, compared to Dapagliflozin-treated PCO group IIb ($p < 0.05$, $P < 0.05$, $P < 0.001$ respectively), and control group I ($P < 0.001$). Concerning Dapagliflozin-treated PCO group IIb, there was a non-significant change in LH ($p > 0.05$) compared to normal control group I. FSH and progesterone levels were significantly decreased in PCO group IIa compared to Dapagliflozin-treated PCO group IIb ($p < 0.05$, $P < 0.001$ respectively), and normal control group I ($P < 0.001$). Also, FSH and progesterone levels were significantly decreased in Dapagliflozin-treated PCO group IIb compared to normal control group ($P < 0.05$).

Table (1): Serum levels of LH, FSH, free testosterone and progesterone in all groups

n=10		Group I Control	Group IIa PCO	Group IIb Dapagliflozin **treated PCO**
LH level (µIU/ml)	**Mean ± SD**	**0.6 ± 0.01**	**0.74 ± 0.03**	**0.58 ± 0.01**
	P of LSD		**P<0.001[a]**	**P> 0.05[a] P<0.05[b]**
FSH level (µIU/ml)	**Mean ± SD**	**2.35 ± 0.13**	**1.11±0.1**	**2.05 ± 0.06**
	P of LSD		**P<0.001[a]**	**P<0.001[a], P<0.05[b]**
Estradiol level(pg/ML)	**Mean ± SD**	**74.6±4.6**	**181.5±5.2**	**101.3±3.1**
	P of LSD		**P<0.001[a]**	**P<0.001[a], P<0.05[b]**
testosterone (pg/ML)	**Mean ± SD**	**46.4±3.6**	**420.80±4.93**	**250.62±3.53**
	P of LSD		**P<0.001[a]**	**P<0.001[a,b]**
Progesterone (ng/ml)	**Mean ± SD**	**4.3 ± 0.11**	**2.23 ±0.13**	**3.3 ±0.21**
	P of LSD		**P<0.001[a]**	**P<0.001[a], P<0.05[b]**

[a] versus normal control group, [b] versus PCO control group

Table (2) statistical analysis of fasting serum glucose level, (mg/dL), serum insulin level (µIU/mL), calculated HOMA-IR} among the studied groups. Serum glucose and insulin levels together with calculated HOMA-IR were increased significantly in PCO group IIa compared to Dapagliflozin- treated PCO group IIb (P<0.001), and compared to control group I (P<0.001). While, levels of glucose, insulin, and calculated HOMA-IR in Dapagliflozin-treated PCO group IIb significantly increased compared to normal control group I (P<0.001 P < 0.05, P < 0.01 respectively).

Table (2): Serum levels of glucose and insulin with calculated HOMA-IR in all groups

n=10		Group I Control	Group IIa PCO	Group IIb Dapagliflozin **treated PCO**
Glucose (mg/dL)	**Mean ± SD**	**90.10±6.22**	**140.70± 4.47**	**105.3 ± 3.33**
	P of LSD		**P<0.001[a]**	**P<0.001[a,b]**
Insulin (µIU/mL)	**Mean ± SD**	**7.43±1.02**	**13.62±1.6**	**9.5±2.01**
	P of LSD		**P<0.001[a]**	**P<0.05[a], P< 0.001[b]**
HOMA-IR	**Mean ± SD**	**1.65±0.16**	**4.7±0.3**	**2.47±0.4**
	P of LSD		**P<0.001[a]**	**P<0.01[a], P< 0.001[b]**

[a] versus normal control group, [b] versus PCO control group

Table (3) statistical analysis of ovarian tissue levels of CRP level mg/ml, TNF level and IL6 pg/ml among the studied groups. The value of ovarian CRP, TNF- alpha, IL6 were increased significantly in PCO group IIa compared to Dapagliflozin-treated PCO group IIb (p <0.01), and normal control group I (P< 0.001, P < 0.01, and P < 0.001 respectively). Moreover, there was a significant increase in CRP, TNF alpha and IL6 in Dapagliflozin-treated PCO group IIb compared to normal control group I (P<0.01, P < 0.05, and P <0.001 respectively).

Table (3): Ovarian tissue levels of C-reactive protein level, TNF alpha and IL6 in all groups

n=10		Group I Control	Group IIa PCO	Group IIb Dapagliflozin **treated PCO**
CRP level (mg/ml)	**Mean ± SD**	**0.68±0.05**	**1.5±0.03**	**0.98±0.02**
	P of LSD		**P<0.01[a]**	**P<0.001[a] , P<0.01[b]**
TNF-a level (pg/ml)	**Mean ± SD**	**84±2.6**	**140±3.2**	**103±4.5**
	P of LSD		**P<0.05[a]**	**P< 0.01[a,b]**
IL-6 level (pg/ml)	**Mean ± SD**	**63±7.3**	**110±5.2**	**85 ±11.03**
	P of LSD		**P<0.001 [a]**	**P<0.001[a] , P<0.01[b]**

[a] versus normal control group, [b] versus PCO control group.

Table (4) statistical analysis of ovarian MDA and SOD among the studied groups. In PCO group IIa, value of MDA was increased significantly (p <0.05), value of SOD was decreased significantly (P < 0.05), compared to Dapagliflozin-treated PCO group IIb, and to normal control group I (P< 0.001). Moreover, there was a significant increase in MDA (P<0.05) accompanied by significant decrease in SOD (P< 0.001) in Dapagliflozin-treated PCO group IIb compared to control group I.

Table (4): Ovarian tissue MDA, SOD in all groups

n=10		**Group I Control**	**Group IIa PCO**	**Group IIb** Dapagliflozin **treated PCO**
MDA (ng/ml)	**Mean ± SD**	**32± 3.2**	**59±6.73**	**45±2.8**
	P of LSD		**p<0.05 [a]**	**P<0.001[a] , p<0.05[b]**
SOD (pg /ml)	**Mean ± SD**	**95±2.6**	**33±4.5**	**62 ±6.4**
	P of LSD		**p<0.001[a]**	**p<0.001[a], p<0.05[b]**

[a] versus normal control group, [b] versus PCO control group

Table (5) statistical analysis of relative mRNA expression of arcuate nucleus kisspeptin 1 and GABA B receptor among the studied groups. In PCO group IIa, value of kisspeptin expression was increased significantly (p <0.01), value of GABA B receptor expression was decreased significantly (P < 0.01), compared to Dapagliflozin-treated PCO group IIb. The same opposing results when both were compared to control group I (P< 0.001). Moreover, there was a significant increase in kisspeptin expression (P<0.05) and significant decrease in GABA B receptor expression (P< 0.01) in Dapagliflozin-treated PCO group IIa compared to normal control group I.

Table (5): Relative mRNA expression of arcuate nucleus kisspeptin 1 and GABA B receptor in all groups

n=10 Relative mRNA expression		**Group I Control**	**Group IIa PCO**	**Group IIb** Dapagliflozin **treated PCO**
Kisspeptin 1	**Mean ± SD**	**1±0.08**	**1.4±0.06**	**1.18±0.05**
	P of LSD		**p<0.001 [a]**	**P<0.05[a] , p<0.01[b]**
GABA B receptor	**Mean ± SD**	**1±0.09**	**0.6±0.03**	**0.83±0.02**
	P of LSD		**p<0.001[a]**	**p<0.01[a], p<0.01[b]**

a versus normal control group , b versus PCO control group.

Table (6) represented TGFβ1 Immunolabelling intensity scores. S1 weak expression of TGFβ1, S2 moderate expression of TGFβ1, and S3 strong expression of TGFβ1.

Table (6): TGFβ1 Immunolabelling intensity scores

TGFβ1		**S3**	**S2**	**S1**
	Range	0.25-0.315	0.183-0.249	0.116-0.182
	Mean ±SD	0.279 ± 0.03	0.19 ± 0.02	0.127 ± 0.01
	P of LSD		**P<0.001[a]**	**P<0.001[a] p<0.01**

[a] versus S3, [b] versus S2

Ovarian H&E

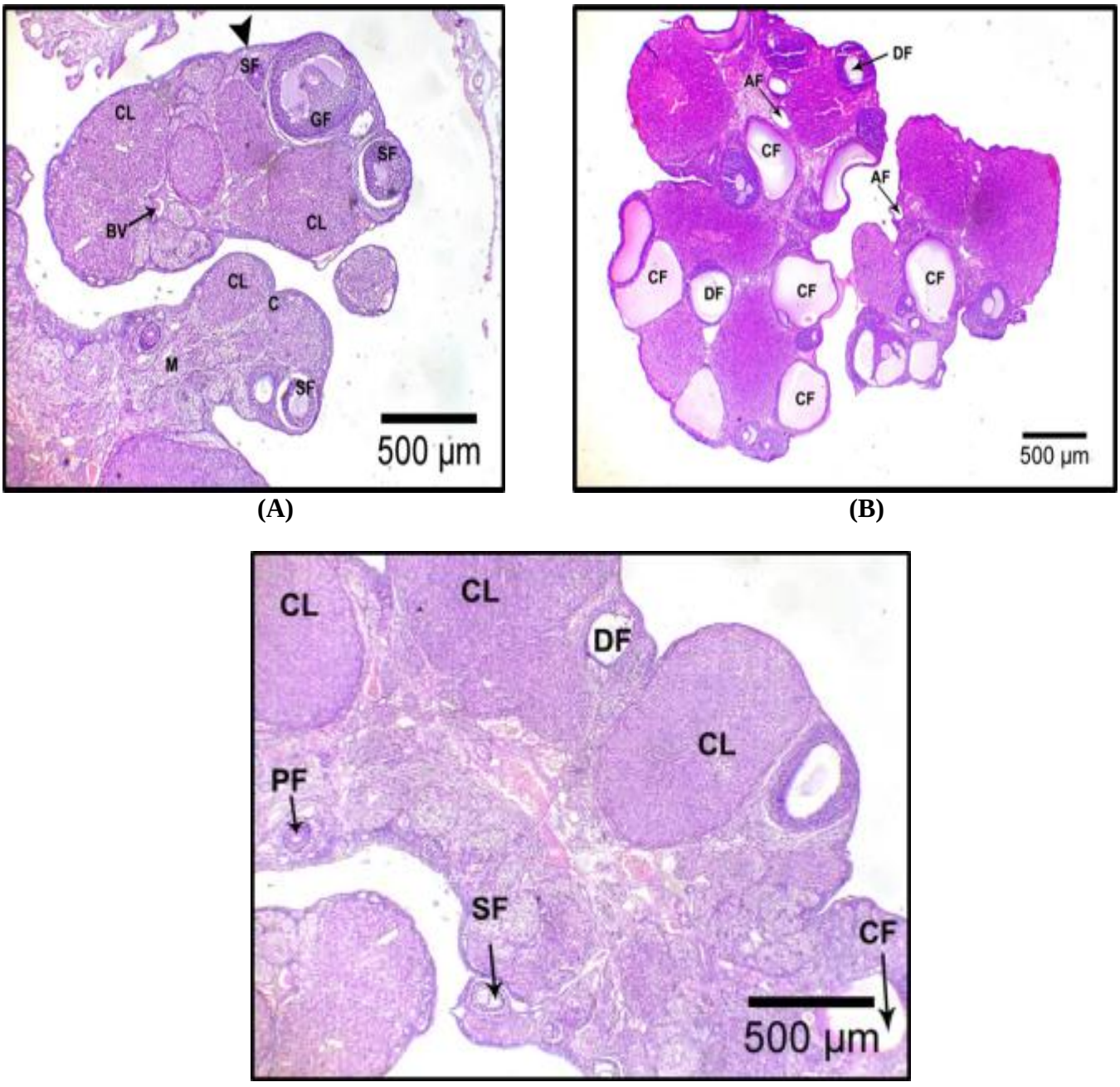
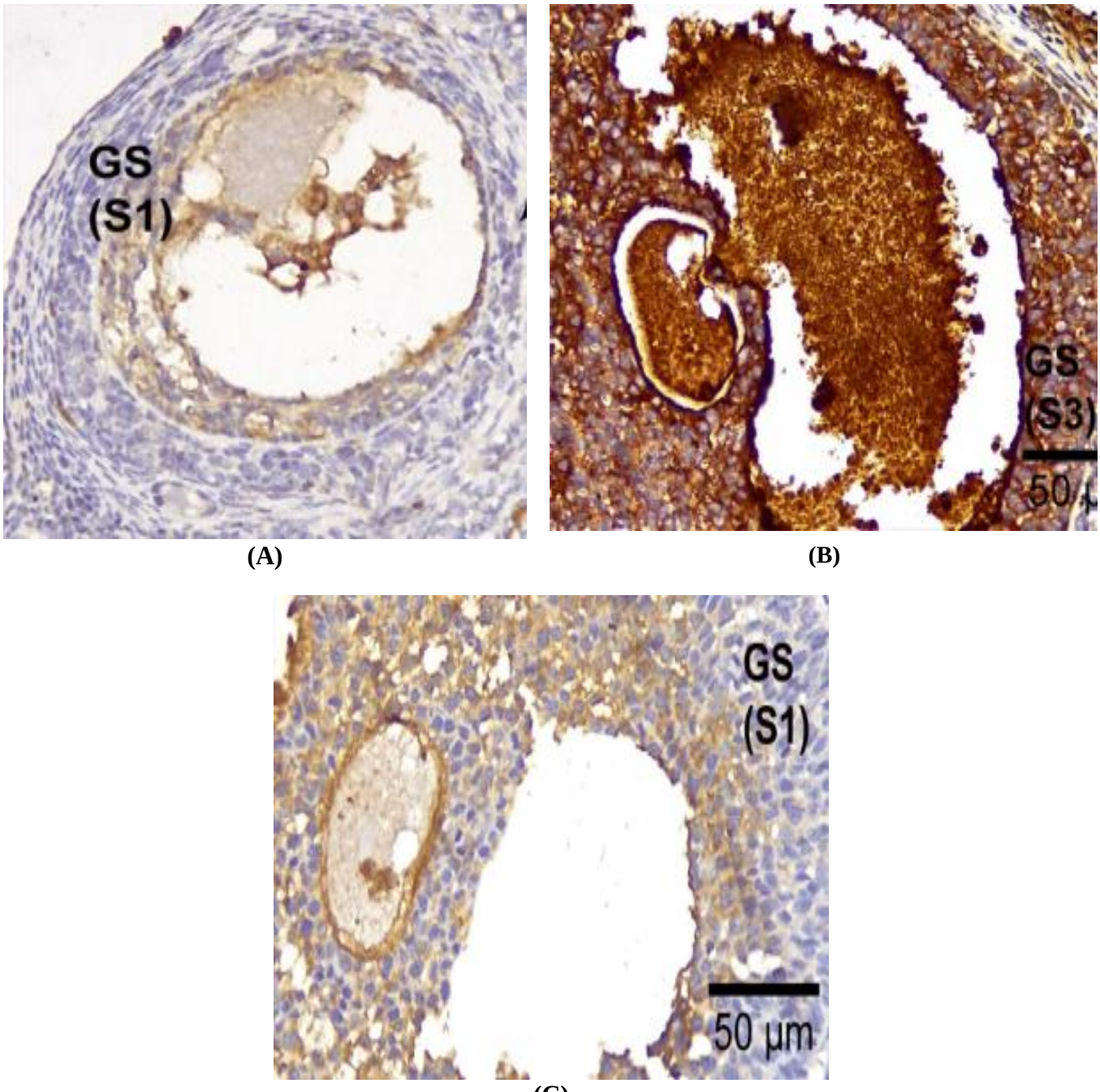


Figure (1): (A): Photomicrograph of ovarian section of control group showed flattened ovarian surface epithelium (arrowhead). It consisted of outer cortex (C) and inner medulla (M). The ovarian cortex revealed various kinds of growing follicles; secondary follicles (SF) with large oocytes, Graafian follicles (GF) and corpora lutea (CL). Notice large blood vessels (BV) in the medulla. (H & E, 40x; Scale bar 500 µm), **(B)** Photomicrograph of ovarian section in PCOS subgroup revealed cystic ovarian follicles (CF), degenerated ovarian follicles (DF) and atretic follicles (AF). With disrupted granulosa cells (H & E, 40x; Scale bar 500 µm), **(C)** Photomicrograph of ovarian section in DAPA-treated PCOS subgroup IIb showed various kinds of growing follicles; primary follicles (PF), secondary follicles (SF), multiple corpora lutea (CL). Few degenerated follicles (DF) and cystic follicle (CF) were still present (H & E, 40x; Scale bar 500 µm).

Ovarian immunohistochemistry



(A) **(B)**

(C)

Figure (2): (A) Photomicrograph of ovarian section of control group showed weak expression of TGFβ1, (GC: granulosa cells, S1: weak). TGFβ1. 400x; Scale bar 50 µm. (B) Photomicrograph of ovarian section of PCOS subgroup showed strong expression of TGFβ1 (GC: granulosa cells, S3: strong). TGFβ1. 400x; Scale bar 50 µm. (C) Photomicrograph of ovarian section of DAPA-treated PCOS subgroup showed weak expression of TGFβ1 (GC: granulosa cells, S1: weak). TGFβ1. 400x; Scale bar 50 µm.

DISCUSSION

In the present study, glucose, insulin and HOMA-IR in PCOS control subgroup were increased significantly. These results are in line with **O'Reilly *et al.*** [14]. In PCOS patients, IR affects phosphoinositide 3-kinase (PI3K) pathway only, but mitogen-activated protein kinase (MAPK) pathway acts in normal way [15]. Hyperinsulinism through MAPK pathway can impair GnRh pulse generator inhibition by progesterone, and increases androgens synthesis in adrenal glands and adipose tissue [14]. Furthermore, hyperinsulinemia inhibits sex hormone binding globulin, so free androgens were increased, and high androgens causes hyperinsulinism, and a vicious cycle between high androgen and increased insulin is continuous [16]. IR may be the result of oxidative stress, which can decrease insulin sensitivity by affecting transduction of insulin signal and decreasing glucose transporter 4 expression on cell surface [17].

In group II b, DAPA decreased serum glucose and insulin, and improved insulin sensitivity. These results agree with **Sinha *et al.*** [18] who stated that DAPA injection for 2 weeks decreased serum glucose, increased insulin sensitivity through increasing glucose uptake by muscle, decreasing glucose formation by the liver, and stimulation of secretion of first-phase insulin pancreatic beta cells.

In PCOS subgroup II a, LH, testosterone, estradiol, and ARC kisspeptin gene expression increased significantly with significant decrease in FSH and progesterone. In subgroup II b DAPA reversed these changes. It is worth noting that serum kisspeptin is increased in PCO patients [19]. Kisspeptins are peptides that are present in neurons of ARC as a neuronal pacemaker, which causes GnRH pulse generation, which has a higher frequency for LH secretion, and a lower frequency for FSH secretion. Also, kisspeptins in ARC mediate negative feedback from estradiol [20, 21]. Kisspeptin is also present in preoptic area and is responsible for estradiol positive feedback to produce L H surge [20]. Hyperinsulinemia in PCOS control group can stimulate kisspeptin gene expression, as insulin crosses BBB, and stimulate kisspeptin ARC secretion through MAPK pathway [22]. In addition, **Nyagolova *et al.*** [19] stated that in women with PCOS, kisspeptin levels are directly associated with fasting insulin and HOMA-IR.

In the present study, DAPA could reduce kisspeptin gene expression, as it improved IR and hyperinsulinemia. Our results are supported by what stated by **Zheng *et al.*** [23] that hypoglycemic drugs can decrease ARC kisspeptin expression in PCOS rats, and there is a problem in the pathway of insulin kisspeptin GnRH in PCOS rat and hypoglycemic drugs may improve this problem.

High kisspeptin increased pulse frequency of GnRH led to increase of LH that caused excess production of thecal androgens. Also, high LH caused an increase in LH/FSH levels, with relative FSH deficiency that causes arrest of follicular growth, polycystic ovarian change, and decreased ovulation that result in progesterone decrease [21]. When the LH/FSH ratio increases, the ovaries increase preferentially the synthesis of estrogen. In addition, high estrogen level is a consequence of anovulation [24]. Dapagliflozin may decrease kisspeptin gene expression by central mechanism, as SGLT2 is expressed in CNS. SGLT2 inhibitors cross the blood brain barrier (BBB), as they are lipid soluble and they have neuroprotective effects in diabetic patients [25]. Another mechanism that can explain how DAPA decrease kisspeptin gene expression through increasing Gamma amino butyric acid (GABA) B receptor gene expression in ARC. GABA is an inhibitory neurotransmitter where **Kamel *et al.*** [26] highlighted the DAPA's anxiolytic effect through GABA B receptor activation. Injection of GABA agonist inhibits kisspeptin neurons and impairs GnRH/LH surge secretion. In addition, GABA release decreases in PCOS. Also, increased kisspeptin expression was increased in ARC of GABA B receptor knock-out mice [27].

Regarding inflammatory status in PCO control subgroup (IIa), C-reactive protein (CRP), TNF-a, and IL-6 were significantly increased, these results agree with that of **Mazloum *et al.*** [10]. These changes were reversed by DAPA. CRP is a liver-derived acute phase protein released by interleukin-6 (IL-6). It is good indicator of low-grade chronic inflammation in PCOS [28].

An increased level of TNF-α can increase theca cells proliferation and cause thickening of ovarian tunica albuginea with subsequent fibrosis. TNF-α inhibits genes involved in production of progesterone, causing reduced ovulation. Moreover, anti-TNF-α antibodies improves follicular development and oocyte meiotic maturation, and decreases granulosa cell apoptosis [29]. Increased IL-6 stimulates inflammation and fibrosis around ovarian tissues and simultaneously causes ovulation failure [30]. DAPA inhibits nuclear factor kappa B (NF-κB) phosphorylation resulting in inhibition of secretion of pro-inflammatory cytokine from endothelial cells and macrophage, and reduced CRP levels in patients with T2D [31]. DAPA also reduce TNF-α and IL-6 in a model of steatohepatitis [32].

In PCO control group (IIa), there were significant increase in MDA, and decrease in SOD. These results agree with **Mazloum *et al.*** [10] who stated that hyperadrenogenic female rats have mitochondrial dysfunction. MDA is Lipid peroxidation product, which accumulate because of intracellular and cell wall damage with increased levels of reactive oxygen species. SOD is an oxygen radical scavenger, which protects cells against oxidative damage.

Dapagliflozin decreased MDA, and increased SOD, DAPA protects proximal tubular cell damage against oxidative stress by decreasing cytosolic and mitochondrial superoxide ROS production. DAPA also disturbs influx of Ca^{2+} by the oxidative-sensitive TRPM2 Ca^{2+} channels [33].

In PCOS ovarian histopathology, there were cystic follicles and degenerated and atretic follicles with disrupted granulosa cells. These results are in line with what stated by **Jashni *et al.*** [34]. In addition, immunohistochemistry showed strong reaction of TGFβ1. On the other hand, DAPA-treated group had a smaller number of degenerated and atretic follicles, and weak reaction of TGFβ1. TGF-β1 regulates protein synthesis, degradation, and apoptosis in extracellular matrix and acts as a potent fibrogenic cytokine, the Smad family are group of proteins that transfer TGF-β1 signaling from the transmembrane receptor to the nucleus [35]. TGF-β1/Smad3 signaling pathway inhibits ovarian follicles development as it enhances granulosa cells apoptosis in PCO rats [35]. Interestingly, DAPA inhibit myocardial fibrosis by suppression of TGF-β1-Smad signaling pathway [8]. In addition, DAPA-treated rats showed a decreased renal expression of TGF-β1 [33].

CONCLUSION

Dapagliflozin improved the hormonal profile, ovarian histopathology and immunohistochemistry in estradiol valerate-induced PCOS. It regulated kisspeptin and GABA B receptor gene expression. It had anti-inflammatory, antioxidant, and anti-fibrotic effects. Therefore, Dapagliflozin could have a future benefit as a therapeutic agent for PCOS.

Financial support and sponsorship: Nil.
Conflict of Interest: Nil.

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