Proficiency of Broccoli (*Brassice oleracea*) Juice in Recovering of Testosterone Andriol-Induced Polycystic Ovary Syndrome Rats

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Abstract

The current study was designed to evaluate the efficiency of broccoli plant in therapy of PCOS that induced for the first time by testosterone andriol (T.A).

Forty-eight immature female rats (21 days old) were divided into 6 groups (8 rats each) as follows: G1, animals were injected with sesame oil for 39 cascade days (control). G2, animals were injected with 1mg/100g b.w of T.A for 39 cascade days. G3, animals were injected with 1mg/100g b.w of T.A gathered with gavaged broccoli juice (b.j) for 39 cascade days. G4, animals were injected with sesame oil for cascade 39 days at the end of last injection were gavaged with d.w for 30 cascade days. G5, animals were injected with 1 mg/100g b.w of T.A for 39 cascade days and at the end of last injection were gavaged with d.w for 30 cascade days. G6, animals were injected with 1mg/100g b.w of T.A for 39 days and at the end of the last injection were gavaged with b.j for 30 cascade days. T.A-induced PCOS rats (G2 and G5) in comparison with controls (G1 and G4) exhibited that had a significant ($P \leq 0.01$) increase in the b.w, ovarian, uterine and fat pads weights as well as the fat cells diameter. Additionally, these groups had acyclicity, high numbers of ovarian cysts, atretic and preantral follicles in the ovaries, lowering the number and the diameter of C.L., and uterine changes. The levels of T, E and LH, an insulin, glucose, LDL, cholesterol in the serum were increased whereas the levels of FSH, LDH were decreased.

Therefore, T.A PCOS model could be appropriated for studied all the features of syndrome i.e, ovarian, metabolic and endocrinologiccal disturbances. Whereas all these measurements of G3 and G6 were ameliorated and recovered into their normalcy. Our findings have shown the benefit of consuming broccoli in the protection (G3) and prevention (G6) of PCOS.

Key words: broccoli, PCOS, rats, andriol-induced

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1.Introduction

Polycystic ovary syndrome (PCOS) was discovered by Stein and Leventhal at 1935, so it was called Stein-Levenathal syndrome [1]. PCOS is the common reason of the infertility in the women of reproductive age [2]. In addition, PCOS possesses a complex health symptom as hyperandroginemia, ovarian cysts, and chronic anovulation [3], amennorrhea, hirsutism, diabetes and obesity, insulin resistance and dyslipidaemia [4], So this syndrome may augment the risk of endometrial and breast cancer [5]. It is well known that, the etiology of this syndrome is unclear. However, there is consensus that refers to the androgen excess, insulin resistance and gonadotropin dynamic are the underling defects in PCOS [6]. There is no single treatment to cure the PCOS because it has several different symptoms and causes which responsible for arising it [7]. Putative drugs were involved in the treatment of PCOS like, metformin, ketoconazole [8], cyprotorone acetate, flutamide [9]. Rai *et al.* [10] indicated to the benefit of melatonin as cure for PCOS. Whereas Yuan *et al*. [11] reported that the BAt-induced diponectin protein will be a new approach in curing of PCOS. These pharmacotherapies were used to manage just some features related with PCOS, either hyperandrogenism and restor ovulation or inhibiting of androgen action or curing of hyperinsulinemia or treatment of hirsutism and acne or for reduction of LDL and increment of HDL. Several serious side effects as severe liver toxicity [8], retention and breast tenderness [9] were seamed with long traditional pharmacological programmes which made them unapproachable. Surgically methods were also used for PCOS therapy. Some kinds of plants were used across varied cultures for medicinal purpose because of their safe and devoiding of the side effects. So they can use an alternative treatment for drugs, surgery, and herbal treatments. Broccoli plant (*Brassica oleracea*) belongs to the family Brassicaceae. It is consumed widely in some areas of the world, due to it is possessing wide therapeutic abilities for several diseases [12]. Broccoli is called crown jewel of nutrition, due to containing numerous health benefit compounds [13]. Numerous bioactive constituents as flavonoids and hydroxylcinnamoyl [12], glucosinolates and isothiocyanate i.e, sulforaphan, indole -3-carbinol (13c), and diindolyl methane (DIM) [14], phenol [15] of broccoli were identified. The previous studies reported that broccoli is the excellent source of minerals such as Ca, Na, Mg, K and trace elements like Fe, Zn, Mn and Cu in addition to vitamins such as C, B and E [16]. It is known that Broccoli has therapeutic properties, like a high antioxidant activity that related to a wide range of its compounds i.e, total phenolic and flavonoids and their derivatives [15-16]. Several studies have shown that broccoli has the protective impact for risk of bladder, prostate breast and colon cancer [17-18]. As well as broccoli is effective in reducing of cholesterol levels [19]. So it has diinodlymethane (DIM) compound that discriminated with antiandrogenic properties which used as androgen antagonist for therapy of prostate cancer [14]. The goal of this study was to determine the impact of broccoli plant in treatment T.A-induced PCOS in rats, as trying to find a safe and alternative therapy of PCOS with minimal side impacts instead of medicinal and surgical methods.

2.Materials and Methods

For obtained immature study female rats at 21 old days (30-32g) that required for achievement our study, 20 adults mature female and 10 male rats of Sprague Dawely, weighting about 170-200g, were mated. All animals, parents and their offsprings were maintained under the condition of controlled lighting (14L: 10 D) and temperature (23-25°C). Food and water were

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available *ad libitum*. This study was conducted in the animal house of College of Education for Pure Science / Ibn Al-Haitham, University of Baghdad.

2.1. Preparation of Hormone

Testosterone andriol (T.A) capsules 40mg (Testo caps) Calalent company was used. For preparing 1mg/100g b.w concentration of T.A, the content of capsule was added to 4ml of sesame oil. The injected volume of A.T 1mg/100g b.w was done according to [20]. This volume was ranged from 0.02-0.2ml depending on the b.w of rats along term of injection (39 days).

2.2. Preparation of broccoli juice (b.j)

Five hundred grams of clean, fresh and sliced broccoli was mixed in a blander and filtered by clean gauze. Animals were orally administrated with b.j at dose 7 mg/kg b.w. This dose was used according to [51] . Freshly juice was prepared every week and kept at 4°C.

2.3. Experimental design

An animal model of PCOS was induced by using testosterone andriol (T.A). Prepubertal female rats at 21 old days were subcutaneously injected and divided into 6 groups (n=8) as follows:

Group1: Female rats were injected with sesame oil (Vehicle) for 39 cascade days.
Group2: Female rats were injected with 1mg/100g b.w of T.A for 39 cascade days.
Group3: Female rats were injected with 1mg/100g b.w of T.A gathered with gavaged b.j for 39 cascade days.
Group 4: Female rats were injected with sesame oil for 39 cascade days, and at the end of the last injection were gavaged with d.w for 30 cascade days.
Group 5: Female rats were injected with 1mg/100g b.w of T.A for 39 cascade days and at the end of the last injection were gavaged with d.w for 30 consecutive days.
Group 6: Female rats were injected with 1mg/100g b.w of T.A for 30 cascade days and at the end of the last injection were gavaged with b.j for 30 consecutive days.

The vaginal smear was taken daily from 45 days until the end of experiments. At the subsequent day of the last treatment, i.e, 61 and 91 days, the animals of all groups were weighted, and the blood was extracted from the heart. Ovaries, uteri and their fat pads were removed and individually weighted. At autopsy of these organs fixed in Bouin's solution. Paraffin sections of ovaries, uteri and fat pads were cut longitudinal and transverse at 5 µm by using routine histological technique and stained with hematoxylin and eosin, for microscopic evaluation [52]. Blood was collected from the animals by heart puncture and allowed to clot for 2h. Serum was extracted after centrifugation for 10 minutes at 3000 rpm and kept at -20°C for hormonal and biochemical analysis.

2.4. Hormonal and biochemical assays

Serum concentrations of T, P, E, FSH and LH were determined in duplicated samples by using automated instrument TOSOH and the TOSOH Bioscience company kits (measuring T, P, E, FSH, LH and insulin) as well as immunoenzymometric assay (IEA). In order to measure insulin, glucose, cholesterol, HDL and LDL automated instrument TOSOH, specific kits Bioscience and Enzymatic colorimetric assay (ECA) were used.

2.5. Statistical analysis

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Statistical analysis was carried by using one-way ANOVA analysis in SPSS (v.11.5) (IBM SPSS static software). All results were represented as mean ± SE. A value of $P \leq 0.05$ was considered to be significant.

Results and Discussion

In the current study, we obtained PCOS model symptoms by administration of T.A to female rats (21 days old) for 39 days (G2 and G5). PCOS symptoms were similar to those of previous rodent models that using other substances and programs [21]. These symptoms involved several features i.e, reproductive, metabolic and endocrinological abnormalities, which associated with human PCOS. But, our study elucidated that the uterine morphological changes could be also additive to the PCOS symptoms. And it has established for the first time to investigate the benefit of broccoli plant for treatment of PCOS symptoms.

3.1 Weight changes and b.j effects

Our data shown in Table 1, indicated that the mean of body weights in T.A- induced rats (G2 and G5) was higher significantly ($P \leq 0.01$) than those of G1 and G4 (controls). So this increment was accordant with the results of Johansson *et al*. [4] and Wu *et al*. [37] who used DHT for PCOS induction. This increment of b.w could be related to the significant ($P \leq 0.01$) elevation in uterine and fat pad weights as evidenced in (Table 1). Additionally, it may be attributed to the increase of body muscles, according to Manneras *et al*. [23] who obtained an increase in the muscle weights as a result to androgen levels elevation in the DHT-induced PSOC rats. For an increase in uterine weights in G2 and G5 may be resulted by the elevation in the estrogen levels (Table 1).

This result was in agreement with the previous study of Kirkland *et al*. [24] who reported that estrogen had a role in the induction of mitotic division of different types of uterine cells. Whereas, this study showed a significant ($P \leq 0.05$) decrement in the mean of ovarian weights of T.A-treated rats (G2 and G5) compared to controls (G1 and G4). This reduction could be attributed to the declining in the number of corpora lutea (C.L) as that observed in the histomorphological of this study. This explanation was agreed with the study of Malven [25] who mentioned that corpora lutea were formed 90% of the ovarian volume. This decrement in the ovarian weight in this study was compatible with the study of Johansson *et al*. [4] who recorded the decrement in ovarian weight of DHT-treated rats. The T.A-treated rats (G2 and G5) showed the irregularly and then stopping of their cyclicity at estrus phase as documented by vaginal smear when compared to controls (G1 and G4) (Table 1).

This result was consistent with Manneras *et al*. [23] and Wu *et al*. [22] who recorded the similar disturbance in the estrus cycle of POCS rats induced with DHT. It was known that C.L via its progesterone secretion was accountable for length and the frequency of estrus cycle of rats [26]. Therefore, the decline in the number of C.L and its secretion: progesterone levels (Table 3 and 5) may cause acyclicity and stopping at estrus phase.

Whereas, our findings showed that, the administration of broccoli juice (b.j.) together with T.A treatment (G3) or post the extreme of T.A treatment (G6) had a positive role in the management of all above disturbance parameters. This role was characterized by a significant ($P \leq 0.01$, $P \leq 0.05$) decrement in the mean weight of the body, fat pads, and uterine weight. A significant ($P \leq 0.05$) increased in ovarian weights was observed. As these values were approximated to those in controls (G1 and G4).

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The administration of b.j caused the cyclicity restoration in (G3 and G6) compared to controls (G2 and G5) (Table 1).All these improved findings may be as a reflection to the declining in T levels and restoring of other hormones to normal levels (Table 5).

Table (1): Effect of broccoli juice in the body weight, reproductive organs, fat pads, and estrus cycle in experimental groups of albino rats

Experimental groups	b.w. (g)	⊠ Ovarian weight (mg/100gb. w)	⊠ Uteri weight (mg/100gb.w)	Fat pads weight (g)	⊠⊠ Regularity of estrus cycle
G1 Treated with Sesame oil	a 143.88 ± 8.43	a 36.87 ± 2.36	a 121.60 ± 9.41	a 1.55 ± 0.13	+
G2 Treated with T.A	b 234.67 ±13.65	b 15.03 ± 2.24	b 198.61 ± 19.5	b 8.84± 1.80	–
G3 Treated with T.A + b.J.	a/c 145.41 ± 4.61	a/c 21.69± 1.42	a/c 145.38 ± 3.55	a/c 1.60 ± 0.14	+
G4 Treated with water post the end of treated with Sesame oil	d 140.80 ± 8.40	d 38.80 ± 2.40	d 119.60 ± 9.21	d 1.25 ± 0.11	+
G5 Treated with water post the end of treated with T.A	e 232.60 ±13.68	e 14.01 ± 2.26	e 199.60 ± 19.11	e 6.85 ± 1.88	–
G6 Treated with b.J. post the end of treated with T.A	d/f 144.92 ±13.94	d/f 22.89 ± 1.59	d/f 164.69 ± 2.58	d/f 1.19 ± 0.16	+

⊠ Values represent the mean ±S.E.
⊠⊠ (+) represent the regular estrus cycle and (-) irregular estrus cycle.
Vertical different letters indicate significant difference (P≤0.01, P≤0.05) between groups.
Vertical similar letters indicate non-significant difference (P>0.05) between groups.

3.2. Histomorphological of POCS rats reproductive organs and b.j effect:

Our results have shown that ovaries of PCOS rats model (G2 and G5) had a significantly (P≤0.01) elevation in the mean numbers of cystic follicles, preantral and antral follicles.

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Additionally, in G2 and G5 a significant (P≤0.01) reduction in the number of normal mature follicles was observed in comparison with controls (G1 and G4) (Table 2, Figure 2, 5, 1 and 4). These results represent the ovarian failure in a selection of the dominant follicle. Thus the high number of preantral follicles in G2 and G5 indicated to stop of follicular development. These results were compatible with many previous reports such as Beloosesky *et al*. [27] who noticed the appearance of cystic follicles with a large number of preantral follicles in Tp-injected female rats. Also, Manneras *et al*. [23] observed a large number of cystic follicles in DHT-treated female rats. As well as, our finding was comported with Wu *et al*. [22] who recorded a significant elevation in the number of cystic follicles, preantral, atretic follicles and disappearance of C.L in TP-treated female rats for 28 days. Causes of these results may be ascribed to the elevation in each of serum T and LH levels as we recorded in our study (Table 5). In the previous study, Gervasio *et al.* [28] reported that the elevation of T levels in ovarian microenvironment has passive effect in the follicular development. On the other hand, the elevation of LH levels in this study could be subserved the follicular recession in an early stage of development, and meanwhile, inhibited the development of dominative follicle resulting to the apparition of cystic follicles, anovulation hence infertility. As well as we suggested that, the defect impact of the elevated T levels on follicular development and ovulation may be due to the induction damage of follicular cells and their organelles membranes which mediated by increased lipid peroxidation. This explanation was similar to the observations of Rezvanfar *et al*. [29] who found that the increment in T hormone levels led to augment the stress oxidation in the ovarian cells. This follicular disturbance in the ovaries of G2 and G5 was a cause for the significant (P≤0.01) reduction in the mean numbers of C.L compared to controls (G1 and G4) (Table 2). This finding referred to the negative impact of elevated T level in the ovulation process followed by clear regression in the numbers of C.L. But the administration of our PCOS rat model with broccoli juice caused apparent improvement in the ovarian function i.e, it has supporting role in the follicular development and ovulatory process, so led to disappearance of cystic follicles. Broccoli juice plus the injection (G3) or post the end of injection (G6) with T.A hormone resulted in a significant (P≤0.05) reduction in the number of cystic follicles, atretic and preantral follicles. Additionally, it caused a significant (P≤0.01, 0.05) increment in the mean number of normal mature follicles and C.L compared to controls (C2 and C5) (Table 2, Figure 3, 6, 2 and 5). These results were coincident with the resumption of hormonal levels i.e, E, T, P and LH into the normal levels (Table 5).

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Table (2): Effect of broccoli juice in the number of different types of ovarian follicles and C.L in experimental groups of albino rats.

Experimental groups	No. of cystic follicles	No. of mature follicles		No. of preantral follicles	No. of antral follicles	No. of C.L
		normal	atretic			
G1 Treated with Sesame oil	a 0.88 ±0.29	a 6.63 ± 0.62	a 2.63 ± 0.49	a 7.75 ± 0.55	a 5.75 ± 0.97	a 11.50 ±0.77
G2 Treated with T.A	b 8.50 ± 0.50	b 4.00 ± 0.46	b 6.11 ± 0.45	b 12.00 ± 0.90	b 11.23 ± 0.55	b 2.00 ± 0.37
G3 Treated with T.A + b.J.	a/c 4.50 ± 0.42	a/c 4.50 ± 0.32	a/c 3.13 ± 0.39	a/c 9.03 ± 0.32	a/c 6.15 ± 0.46	a/c 6.00 ± 0.32
G4 Treated with water post the end of treated with Sesame oil	d 0.82 ± 0.20	d 7.60 ± 0.69	d 2.33 ± 0.40	d 6.55 ± 0.56	d 4.05 ± 0.98	d 13.61 ±0.80
G5 Treated with water post the end of treated with T.A	e 7.59 ± 0.55	e 3.01 ± 0.40	e 5.70 ± 0.41	e 12.55 ± 0.95	e 10.07 ±0.41	e 1.51 ± 0.11
G6 Treated with b.J. post the end of treated with T.A	d/f 3.00 ± 0.32	d/f 6.51 ± 0.57	d/f 2.75 ± 0.31	d/f 9.25 ± 0.90	d/f 6.15 ± 0.45	d/f 10.88 ±0.39

Values represent the mean ±S.E.
Vertical Different letters indicate significant difference (P≤0.01, P≤0.05) between groups.
Vertical similar letters indicate non-significant difference (P>0.05) between groups.

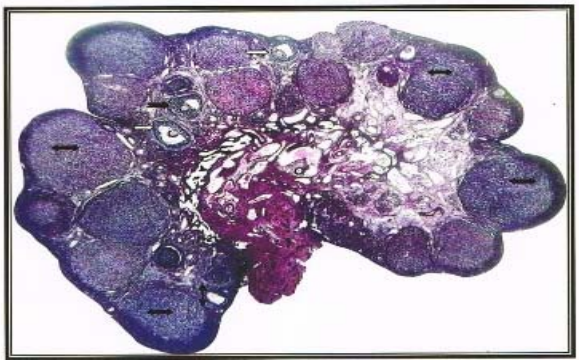

Figure (1): Longitudinal section (L.S) of ovary from G1 (control) showing normal follicle (⇨), C.L (⬌), cystic follicles (⟷) antral follicle (➡) (H&E, 3.2x).

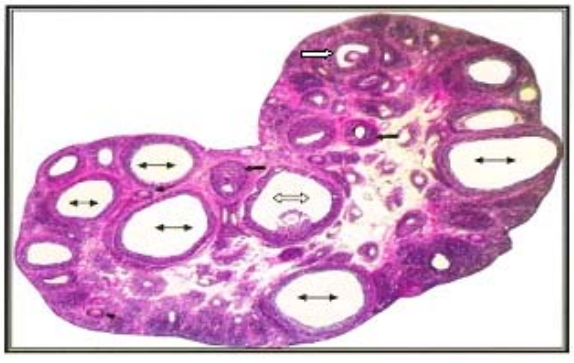

Figure (2): L.S of T.A-Treated rats ovary (G2), cystic follicle (⟷), antral follicle (⬅), preantral follicle (←), atretic follicle (⇔),normal follicle (⇨), with disappearance of C.L (H&E, 3.2x).

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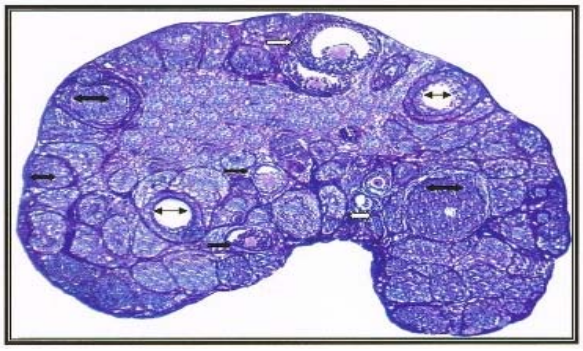

Figure (3): L.S of ovary from T.A-treated rats with administration of b.j. (G3), showing regression in cystic follicle (⟷), atretic follicle (⇔), antral follicle (➡), and appearance normal follicle (⇨), with large of C.L (⬌) (H&E, 3.2x).

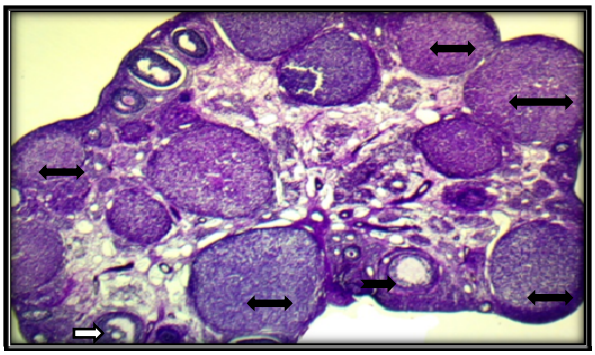

Figure (4): L.S of ovary from G4 (control) showing normal follicle (⇨), C.L (⬌), antral follicle (➡),(H&E, 3.2x).

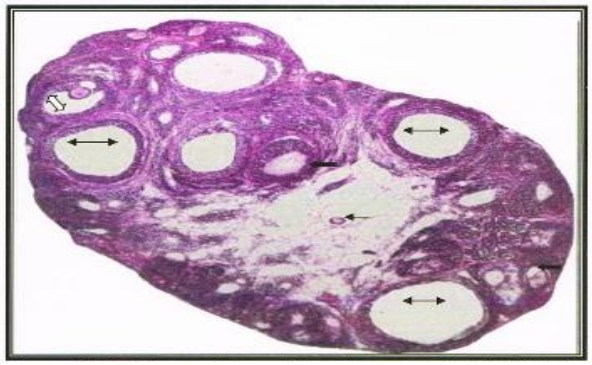

Figure (5): L.S of ovary from T.A-treated rats administrated with water at the end of T.A injected (G5), showing cystic follicle (⟷), antral follicle (➡), preantral follicle (←), atretic follicle (⇔), with disappearance of C.L (⬌), (H&E, 3.2x).

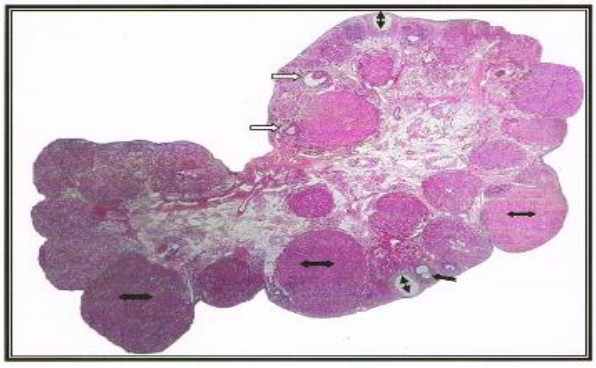

Figure (6): L.S of ovary from T.A-treated rats administrated with b.j. at the end of AT injected (G6), showing regression in number of cystic follicle (⟷), antral follicle (➡), with restore of normal follicle (⇨), and C.L (⬌) (H&E, 3.2x).

3.3. Effect of b.j. on the diameter and thickness of ovarian follicles and C.L.

The current results showed that the injection of T.A hormone (G2 and G5) caused a significant (P≤0.05, 0.01) increment in the mean of cystic follicle diameters and theca layer thickness, while granulosa layer thickness was significantly (P≤0.01) reduction compared with those of G1 and G4(Table 3, Figure 2, 5, 1 and 4). However, in normal mature follicles, the diameter and thickness of granulosa layer showed a significant (P≤0.01) reduction, while a significant (P≤0.01) increment in the thickness of theca layer was recorded in comparison with (G1 and G4). Additionally, in G2 and G5 the C.L diameters were significantly (P≤0.01) decrement compared to those of controls (G1 and G4). The elevation in thickness of follicle's theca layer and reduction

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in the granulosa layer might be explained as a reflection of the increasing T levels in serum. It was reported that hyperplasia in the theca layer and ovarian tissue resulted in an increase in the cellular proliferation that companies with granulosa layer apoptosis by hypersynthesis of androgen [30]. Ortega *et al.* [31] also mentioned the ovarian hyperandrogenism leading to stimulate of androgen receptor in the thecal layer, which induces of anabolic effect and caused thecal layer proliferation. Baravalle *et al*. [32] reported that the reduction in the thickness of granulosa layer of cystic follicles may be due to apoptosis and the growing cells became similar to epithelial cells and forming epithelium at the basal layer of granulosa. The present findings were in agreement with the study of Manneras *et al*. [23] who noticed that ovaries had cystic follicles with thickened thecal layer and reduction in the granulosa layer in DHT PCOS rats model. Also, the lowering of the diameters of C.L in this study may be due to the disturbance of follicular development leading to anovulation. While the administration of b.j in T.A treated rats (G3) led to improvement the ovarian morphology and function. Table 3 showed that a significant ($P\leq0.01$) reduction in the diameter of cystic follicles and a significant ($P\leq0.01$) elevation in the diameter of normal follicles and C.L. We have observed that a significant ($P\leq0.05$) decrement in the thickness of cystic follicle theca layer with a significant ($P\leq0.05$) elevation in the thickness of cystic and normal follicle granulosa layer when compared with G2. While nonsignificant ($P>0.05$) in the mean of diameter and thickness of follicles and C.L were observed in comparison with G1. When the administration of b.j post the ending of the treatment with T.A (G6) caused a significant ($P\leq0.01$) reduction in the mean of cystic follicle's diameter and its theca layer thickness. While its thickness of granulosa layer was significantly ($P\leq0.05$) higher than control (G5). In addition, a significant ($P\leq0.05$) increase in the diameters of normal follicles and thickness of granulosa layer, and a significant ($P\leq0.01$) decrement in the thickness of theca layers were recorded. As a significant ($P\leq0.05$) elevation was observed in diameters of C.L than G5. But there was no difference ($P>0.05$) concerning with diameter and thickness of follicles and C.L for G6 than G4. Interestingly, the impact of T.A hormone treatment (G2 and G5) had not only induced PCOS traits i.e, ovarian, metabolic and endocrinological symptoms but accompanied with uterine changes. So it caused a significant ($P\leq0.01$) increment in the mean of diameters and thickness of endometrium i.e, "epithelium and lamina properia" as well as myometrium than controls (G1 and G4) (Table 4, Figure 8, 11, 7 and 10). Also, the involvement of T hormone in the induction of the increment of uterine development may be via its indirect role i.e, T conversion to estrogen hormone by aromatization, then E_2 caused the appearance of uterine anabolic effects. We could suggest that T hormone had a direct anabolic effect on the myometrium. This result was in accordance with Zhang *et al*. [33] who indicated that the injection of 6 mg/100 g of DHEA hormone into female rats for 35 days caused the increase in the myometrium. In the current study, fat cell diameters were significantly ($P\leq0.01$) elevated for G2 and G5 compared with G1 and G4 (Table 4, Figure 14, 17, 13 and 16). This finding may be yielding about the disturbance of metabolic lipid as a result to the elevation of T levels in this study. Moverare-Skirtc *et al*. [34] reported the increment in lipid mass of hysterectomy male mice by activation of androgen receptor via DHT injection. Our result was coincident with Manneras *et al*. [23] who recorded the increment in fat cell volume in DHT-induced PCOS rat. As Beloosesky *et al*. [27] indicated that the injection of TP in female rats caused obesity. And the injection of DHT caused by the obesity in women [35]. Post the administration of b.j either with T.A injection or at the end of T.A injection (G3 and G6), positively improved all the studied

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uterine parameters and fat pads compared with G2 and G5 (Table 4, Figure 9, 12, 8, 11, 15, 18, 14 and 17).

Table (3): Effect of broccoli juice in the diameter and thickness of ovarian follicles wall and C.L in experimental groups of albino rats

Experimental groups	Cystic follicles			Normal of mature follicles			C.L
	Diameter (µm)	Thickness (µm)		Diameter (µm)	Thickness (µm)		Diameter (µm)
		Thecal layer	Granulosa layer		Thecal layer	Granulosa Layer	
G1 Treated with Sesame oil	a 255.40±44.83	a 3.90±0.24	a 3.44 ±0.27	a 581.67 ±4.33	a 1.44 ±0.06	a 5.23 ±0.05	a 768.50 ±24.53
G2 Treated with T.A	b 477.72 ±26.73	b 4.25 ±0.19	b 2.20 ±0.32	b 227.23 ±5.43	b 2.85 ±0.13	b 3.00 ±0.06	b 189.47 ±4.33
G3 Treated with T.A + b.J.	a/c 268.31 ±3.15	a/c 3.15 ±0.22	a/c 3.00 ±0.33	a/c 451.16±11.48	a/c 2.20 ±0.07	a/c 3.84 ±0.06	a/c 233.20 ±6.93
G4 Treated with water post the end of treated with Sesame oil	d 250.49 ±43.63	d 4.40 ±0.27	d 2.41 ±0.37	d 578.97 ±4.37	d 1.45 ±0.09	d 6.20 ±0.08	d 760.57 ±24.49
G5 Treated with water post the end of treated with T.A	e 475.71 ±26.70	e 5.20 ±0.14	e 1.22 ±0.30	e 220.20 ±5.46	e 2.00 ±0.17	e 4.02 ±0.07	e 185.45 ±4.31
G6 Treated with b.J. post the end of treated with T.A	d/f 344.50 ±13.98	d/f 4.09 ±0.15	d/f 2.08 ±0.19	d/f 487.45 ±36.15	d/f 1.57 ±0.11	d/f 4.87 ±0.33	d/f 561.13 ±80.86

Values (µm) represent the mean ±S.E.
Vertical different letters indicate significant difference (P≤0.01, P≤0.05) between groups.
Vertical similar letters indicate non-significant difference (P>0.05) between groups.

Table (4): Effect of broccoli juice in the diameter and thickness of uterus layers and Fat cells in experimental groups of albino rats.

Experimental groups	Uterus diameter (µm)	Thickness of endometrium (µm)		Thickness of myometrium (µm)	Diameter of fat cells (µm)
		Epithelium	Lamina properia		
G1 Treated with Sesame oil	a 1547.43 ±56.70	a 12.58 ±0.96	a 244.55 ±8.36	a 234.52 ±5.81	a 3.40 ±0.21
G2 Treated with T.A	b 1788.75 ± 32.67	b 37.07 ±4.47	b 352.45±40.20	b 392.52 ± 22.58	b 7.33 ± 0.15
G3 Treated with T.A + b.J.	a/c 1351.62 ±10.98	a/c 13.25 ± 1.00	a/c 253.07 ± 7.87	a/c 249.10 ± 16.51	a/c 4.51 ± 0.11
G4 Treated with water post the end of treated with Sesame oil	d 1540.41 ± 54.72	d 15.25 ±1.57	d 240.45 ±8.86	d 229.50 ±5.71	d 3.38 ±0.20
G5 Treated with water the post end of treated with T.A	e 1780.76 ±32.60	e 39.56 ±2.17	e 358.40±40.26	e 397.59 ±22.51	e 8.30 ±0.11
G6 Treated with b.J. post the end of treated with T.A	d/f 1451.25 ±22.38	d/f 16.45 ±1.57	d/f 226.57±10.00	d/f 310.01 ±17.57	d/f 4.61 ±0.20

Values (µm) represent the mean ±S.E.
Vertical different letters indicate significant difference (P≤0.01, P≤0.05) between groups.
Vertical similar letters indicate non-significant difference (P>0.05) between groups.

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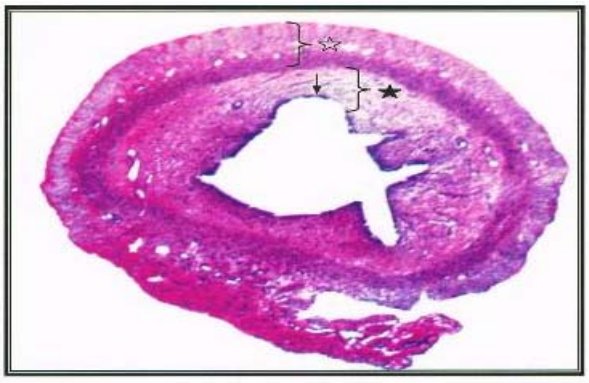

Figure (7): Transverse section (T.S) of uterus from (G1) showing the epithelium (ETM,↓), lamina properia (L.P,★), myometrium (MTM,☆), (H&E, 4x).

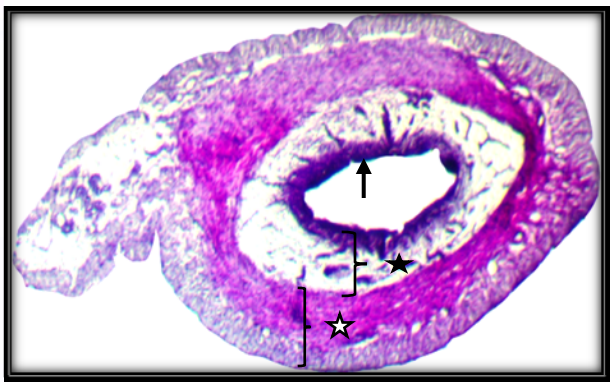

Figure (8): T.S of uterus from (G2) showing (ETM,↑), (L.P,★), (MTM,☆), (H&E, 4x).

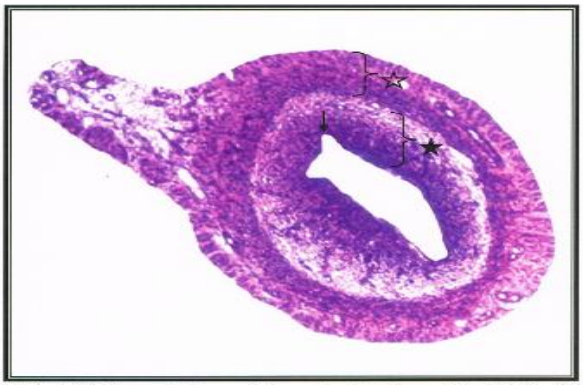

Figure (9): T.S of uterus from (G3) showing (ETM,↓), (L.P,★), (MTM,☆), (H&E, 4x).

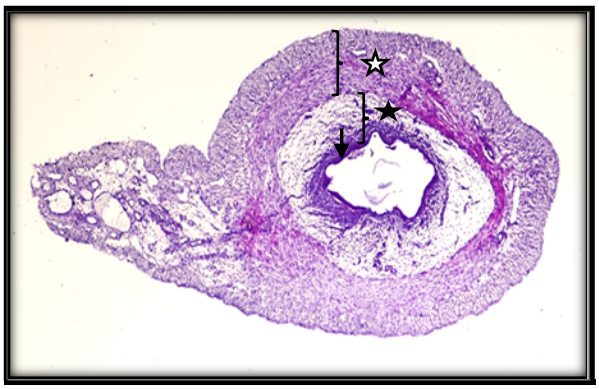

Figure (10): T.S of uterus from (G4) showing (ETM,↓), (L.P,★), (MTM,☆), (H&E, 4x).

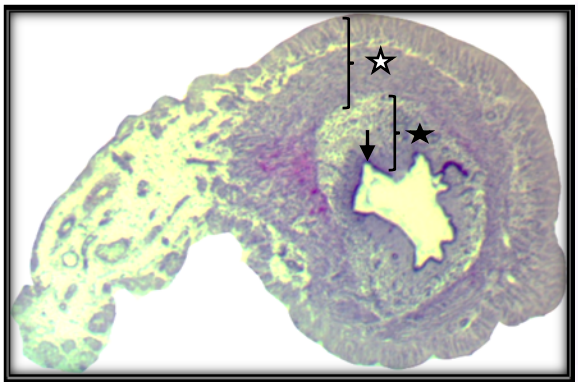

Figure (11): T.S of uterus from T.A-treats rats administration with water at the end of T.A injected (G5), showing (ETM,↓), (L.P,★), (MTM,☆),

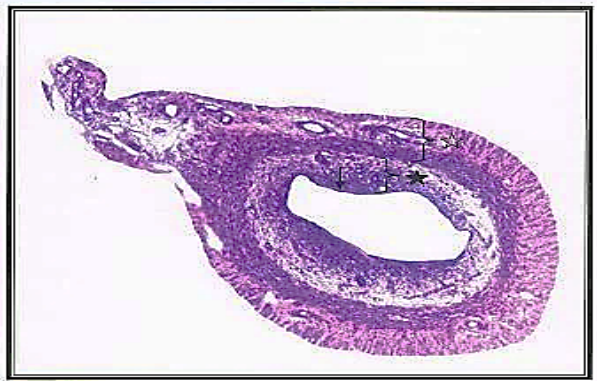

Figure (12): T.S of uterus from (G6) showing (LTM,↓), (L.P,★), (MTM,☆), (H&E, 4x).

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(H&E, 4x).

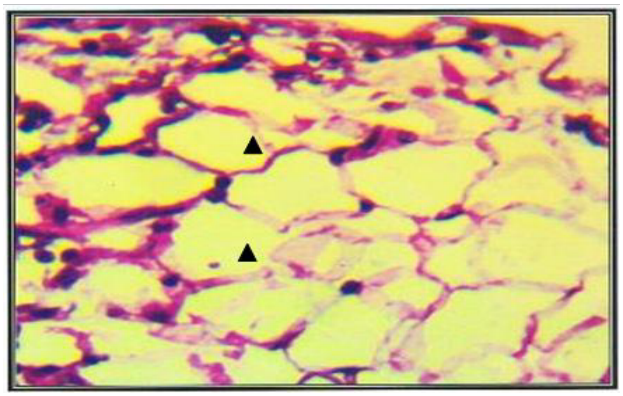

Figure (13): T.S of fat pad (G1) showing fat cells (▲), (H&E, 40x).

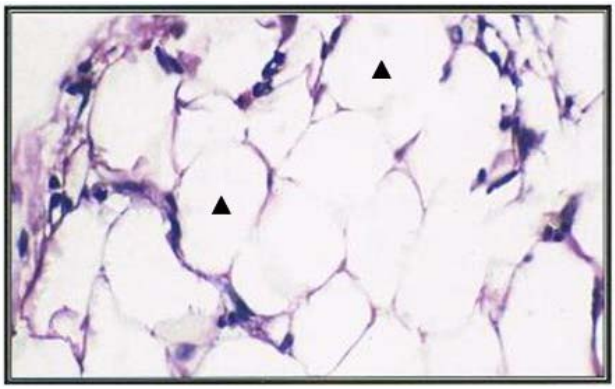

Figure (14): T.S of fat pad (G2) showing fat cells (▲), (H&E, 40x).

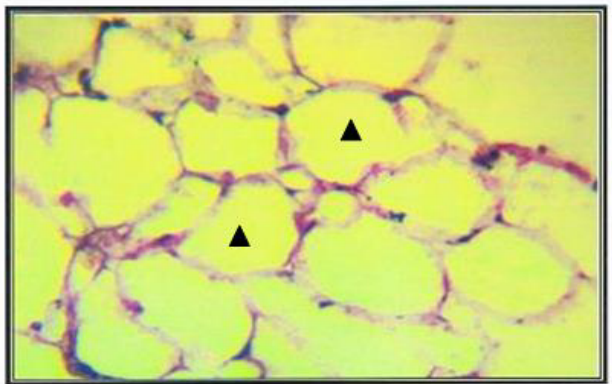

Figure (15): T.S of fat pad (G3) showing fat cells (▲), (H&E, 40x).

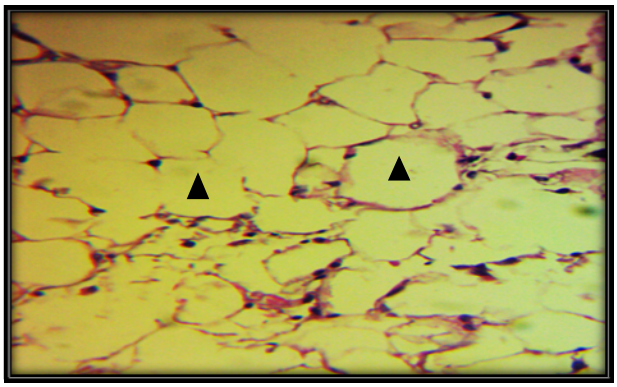

Figure (16): T.S of fat pad (G4) showing fat cells (▲), (H&E, 40x).

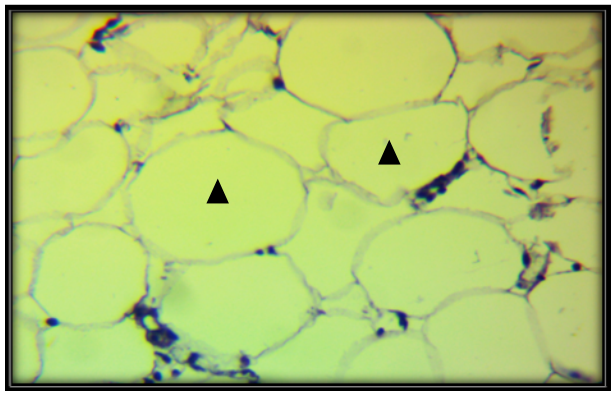

Figure (17): T.S of fat pad (G5) showing fat cells (▲), (H&E, 40x).

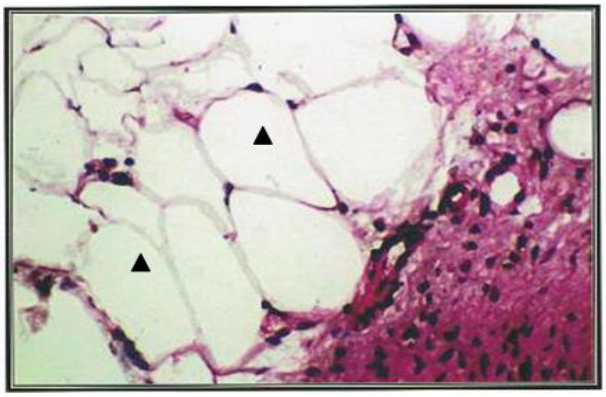

Figure (18): T.S of fat pad (G6) showing fat cells (▲), (H&E, 40x).

3.4. Effect of b.j. on the hormonal and biochemical indicators

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Table 5 showed that the injection of T.A (G2, G5) caused hormonal profile disturbance, representing with a significant ($P \leq 0.01$ and $P \leq 0.05$) elevation in the serum T, LH and E_2 compared to controls (G1 and G4). While it caused a significant ($P \leq 0.01$) decrement in the FSH

and P hormones for G2 and G5 compared with controls (G1 and G4). As the elevation in T levels confirmed the occurrence of PCOS. As previously reported that hyperandrogenism is a major cause for PCOS [3]. The elevation in the T levels was mediated by a significant increase in the thickness ˝the number of its cells" of theca layer that produced ovarian androgen under elevated LH impact. According to Hillier *et al*. [36] who mentioned that, androstenedione is synthesized from the conversion of P hormone to T hormone by 17-hydroxysteroid dehydrogenase in the cell of granulosa layer under LH hormone stimulation. For the elevation of LH hormone in our study could be explained based on that perishable of the effect of E_2 and P negative feedback mechanism on gonadotropic neurons in the hypothalamus, performed to the continuing LH hormone secretion. Whereby Moor [37] confirmed that the elevation of androgen (T) causes the impairment for the P feedback mechanism via lower of progesterone receptors gene expression in paraventricular nuclei of hypothalamus which led to the continuity of GnRH- releasing nuclei to secret fast pulse of GnRH and then continuance of LH releasing that resulting to anovulation. The serum T hormone levels elevation in our study was concordant with cholesterol elevation (Table 5). This result was accordance with the study by Palermo [38] who reported that cholesterol s required requisite precursor molecule for T hormone synthesis. We could suggest that the aromatization process of some T hormone molecules might be a cause for the elevation of E_2 hormone in our study. In the previous study, Hillier *et al*. [36] reported that T hormone was converted to E_2 post its transport into granulosa cells by aromatase enzyme under FSH effect. Decreasing levels of FSH in this study may be due to the fast pulse secretion for GnRH that caused LH elevation. It was well known that FSH secretion required slow pulse secretion of GnRH in the onset follicular development hence ensues of ovulation. The lowering progesterone levels in the current study could be related to the decrement in the mean number and diameter of C.L, that is considered the major source of progesterone production. This result was compatible with Beloosesky *et al.* [27] who reported that confirmed anovulation with failure in P production had shown in TP injected female rats for 35 days. While Manneras *et al*. [23] reported that there was no change in T levels, but just a significantly lower was noticed in P levels of DHT-injected female rats. This discordance may due to the difference in race, the kind of instigated hormone for PCOS, dosage, and programme of injection in our study. A significant ($P \leq 0.01$) elevation was observed in insulin, glucose, cholesterol and LDL in G2, G5 compared to controls (G1 and G4) (seemed Table 5). The metabolic disturbances have confirmed the incidence of PCOS in our programme of T.A injection. Also, it has seemed that these disturbances were associated with high T levels. The elevated insulin levels have indicated to an occurrence insulin resistance. This result could be attributed to abnormal insulin secretion or amiss of its function. Wherein Dunaif [39] indicated that hyperinsulinism included abnormality of internal function (hyperphsphoserine) post insulin correlation with its receptors which led to high serum insulin levels. Our results were consistent with Beloosesky *et al*. [27] who observed insulin resistance after 35 days of T.P injection in female rats. So Manneras *et al*. [23] recorded high insulin resistance in female rats injected with DHT. As it was recorded that similar results in PCOS women after injection with DHT [35]. And our findings were compatible with Ciaraladi *et al*. [40] who mentioned that the body cells of PCOS women were less sensitive to the antilipolytic insulin effect than normal women, while the insulin receptors and kinase activity in fat cell were

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normal in spite of its sensitive reduction to stimulation of glucose transport. Additionally, the elevated insulin in G2 and G5 could be

attributed to the elevated serum T levels. Previous data revealed that T hormone induced insulin resistance via curtailing the presence and competency of specific transporter protein i.e, GLUT4 which responsible for glucose transport, in muscle and fat cells [41]. In the current study, the elevation of LDL and reduction of HDL levels have indicated the disturbance in lipid metabolism that associated with PCOS. The reason of these defects may be due to the increment of serum T levels that caused the increase of enzymes which responsible and involved in the cholesterol biosynthesis pathway, then led to a high of the serum cholesterol and abnormal LDL and HDL levels. It was reported that the women with PCOS demonstrated the perturbation in the fat metabolism of their body fats [42]. Al-Hakeim *et al*. [43] revealed that the increment LDL and decrement of HDL in PCOS women led to cardiac vascular disease. Our result was in agreement with Johansson *et al*. [4] who reported similar result i.e, the elevation in cholesterol and LDL levels in female rats injected with 32 µg. On the other hand, the administration of b.j with T.A injection (G3) or after the end of T.A injection (G6) owns a positive role in improving or adjusted the hormonal and biochemical profile compared with G2 and G5. So we could notice restoration of LH, T, FSH and P nearly normal levels. And the same role of b.j for insulin, glucose, cholesterol, LDL and HDL has detected explanations of the mechanism of b.j against PCOS in our study (Table 5).

Table (5): Effect of broccoli juice in the serum hormonal and some biochemical indictors levels in experimental groups of albino rats

Experimental groups	T ng/ml	LH mIU/ml	FSH mIU/ml	E_2 pg/ml	P ng/ml	Ins Uu/ml	Glu mg/dl	Chol mg/dl	HDL mg/dl	LDL mg/ml
	Mean ±S.E									
G1 Treated with Sesame oil	a 0.99 ± 0.20	a 0.09 ± 0.02	a 0.15 ± 0.02	a 43.65 ± 0.53	a 33.55 ± 4.24	a 0.11 ± 0.03	a 190.62 ± 5.27	a 72.87 ± 3.59	a 24.50 ± 3.24	a 35.62 ± 2.58
G2 Treated with T.A	b 9.89 ± 0.29	b 0.25 ± 0.02	b 0.07 ± 0.02	b 54.40 ± 1.91	b 3.71 ± 0.07	b 0.21 ± 0.03	b 249.87 ± 17.87	b 90.20 ± 5.38	b 18.00 ± 1.77	b 59.50 ± 3.59
G3 Treated with T.A + b.J.	a/c 7.50 ± 0.19	a/c 0.13 ± 0.01	a/c 0.11 ± 0.01	a/c 49.70 ± 0.98	a/c 11.18 ± 0.94	a/c 0.10 ± 0.01	a/c 205.00 ± 0.01	a/c 56.00 ± 0.01	a/c 23.50 ± 1.32	a/c 30.00 ± 1.88
G4	d	d	d	d	d	d	d	d	d	d

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Treated with water post the end of treated with Sesame oil	0.90 ± 0.21	0.08 ± 0.01	0.10 ± 0.02	40.40 ± 0.43	30.48 ± 4.14	0.05 ± 0.01	188.52 ± 5.20	69.42 ± 3.50	26.55 ± 3.28	30.61 ± 2.53
G5 Treated with water post the end of treated with T.A	e 10.80 ± 0.22	e 0.54 ± 0.03	e 0.05 ± 0.01	e 57.41 ± 1.92	e 2.70 ± 0.07	e 0.24 ± 0.04	e 250.08 ± 17.87	e 93.20 ± 5.30	e 15.01 ± 1.78	e 60.51 ± 3.59
G6 Treated with b.J. post the end of treated with T.A	d/f 3.96 ± 0.62	d/f 0.07 ± 0.02	d/f 0.16 ± 0.01	d/f 10.72 ± 0.65	d/f 7.00 ± 0.10	d/f 0.05 ± 0.05	d/f 158.57 ± 5.85	d/f 54.00 ± 0.01	d/f 23.25 ± 0.77	d/f 39.12 ± 2.14
Vertical different letters indicate significant difference (P≤0.01, P≤0.05) between groups. Vertical similar letters indicate non-significant difference (P>0.05) between groups.										

In this study, the curative and protective properties of raw broccoli juice consumption were most likely mediated via its bioactive compounds that induce different physiological functions including controlling feedback mechanism of hormones, regulating of metabolic interactions within the body, acting as antiandrogen and antioxidants. Our findings have suggested that b.j caused a strong inhibition in the progesterone feedback on neurogenic GnRH. However, this inhibition may be resulted by increasing of progesterone receptors expression, then declining frequency of GnRH secretion which led to LH suppression followed by T reduction and restoring of normal FSH levels secretion. Thereby FSH restoration caused cystic, atretic follicles and the number of prenatal and antral follicles regression. So the improvement effect of b.j may cause the reduction LH receptors of theca layer (Major source of ovarian hormone biosynthesis) which leads to demise the supporting effect of LH on the proliferation of follicular wall's theca cell, followed by theca layer thickness reduction and then an occurrence of T level lowering. Hence this lowering was responsible for the abrogate of uterine inversely changes and returning to their negative effect on the included enzymes in the androgen biosynthesis i.e, the enzyme that involves in 17-OH pregnenolone pathway or 17-OH progesterone. Briefly, the effect of b.j may have a role in cancels of a paracrine mechanism of hormone (Stimulation) in the theca cells. In addition, the T levels reduction by b.j. at the present study may be due to well-Known eliminator role of broccoli on cholesterol molecules ˝precursor of T hormone synthesis".

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In addition, Yousef [44] reported that constant cholesterol supply was necessary for T hormone biosynthesis from pregnenolone and androstenedione compounds. The suppression of follicular development and ovulation processes in our PCOS model could be explained depending on the oxidative stress by free radical's level increment as result of elevated T levels. As the previous study confirmed that, the associated hyper androgenism with PCOS caused

increasing of ROS followed by a reduction in ovarian cell function [45]. The improvement by b.j in each of follicular development and ovulation processes in treated groups with b.j (G3 and G6) may be based on, the antioxidant activity of broccoli. As well as broccoli was considered as the scavenger for oxidative substance ´that caused living cell distraction" by its high content phenols, flaphomedies, and vitamins. Wherein Yu *et al.* [46] confirmed that such these compounds had antioxidant activity. Porter [47] studied the activity levels for phenols, flavonoid, and vitamins in broccoli plants and measured it with different methods and ways of cooking. These authors found that the activity of these compounds were higher in raw broccoli than boiling broccoli or cooked broccoli by microwave. For the potency of b.j action in the modulation of metabolic disturbance that associated with PCOS rats in this study could be explained as follows:

The major improvement in insulin and glucose levels in b.j administrating PCOS rats (G3 and G6) may due to the restoring serum T levels and disappearance of its passive effect i.e, β cell damage or internal insulin signal reduction. So b.j may boost the gene expression and transfusion of glucose transporter in the cell and its conductivity into the plasma membrane, that helping the glucose accessing into the cells then followed by restoring of glucose and insulin into normalcy levels. Whereby Johansson *et al.* [4] reported that insulin stimulating the protein gene expression of glucose transporter in all cells. The positive effect mechanism of b.j on the metabolic lipid representing with restoring of cholesterol levels into normality in this study could explain as follows. The positive impact of b.j might be due to the principle component of broccoli plant as sulpheraphane. Suido *et al.* [19] reported that sulpherphane owns high activity for the reduction of cholesterol. It was well known that broccoli considered as a rich plant with soluble fibres that associate with serum cholesterol and facilitating of its excretion or secretion that preformed to declining of serum cholesterol levels.

According to Szalay [48] who reported that broccoli is rich with soluble fibers and causes the declining of cholesterol and LDL at 30% and 40% respectively. The reduction of LDL in broccoli-treated PCOS rats might be due to one component of broccoli i.e, 13C active compound. The previous studies revealed that rich broccoli in 13C will reduce the serum apolipoprotein that preventing or diminishing the synthesizing of LDL cholesterol. It was well known that lipoprotein caused LDL anabolism and transfusion into the different area of the body. However, broccoli plant has a high C vitamin levels that reduce the cholesterol and LDL. Cherney [49] reported that C vitamin has a potency to prevent the formation of free radicals that increase of HDL-cholesterol levels, in addition, broccoli contains β-carotene that possesses antioxidant role as C vitamin which prevents LDL formation. Finally, by using of T.A most of the findings were similar to results of our previous study using other PCOS model, except that relating to the body, ovarian weight, and estrogen levels. So this consequence indicated to the ability of T.A in an induction of all PCOS features. Also, broccoli had the same role in prevention and curing of all those features as in rats with Letrozole-induced PCOS [50].

4. Conclusions

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For the first time, that we obtained many findings represented by 1-T.A had strong effect to induce all symptoms i.e, ovarian, metabolic and endocrinocological features, similar to the human symptoms of PCOS, 2- T.A caused uterine disturbances that could be considered one of the reasons for PCOS symptoms infertility in addition to the known PCOS symptoms, 3- broccoli plant consumption was efficacious to prevent (G3) and corrective (G6) for all PCOS symptoms,

given to its bioactive components. And broccoli had the antiandrogenic impact (via the reduction of T levels), controlling on feedback mechanism and regulating metabolic parameters within the body.

We recommend that women with PCOS should be taken up raw broccoli that has high dietary value, based on our results that revealed b.j was able to improve, curing and prevention of all symptoms of PCOS. Also, broccoli plant is considered as a safe nutrient as it owns other uses.

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