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THERAPEUTIC POTENTIAL OF BETA-CARYOPHYLLENE (BCP) IN POLYCYSTIC OVARIAN SYNDROME (PCOS): INSIGHTS FROM RAT MODEL STUDIES

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ABSTRACT

Beta-caryophyllene (BCP) was investigated for its potential therapeutic benefits on polycystic ovarian syndrome (PCOS), a common endocrine disorder in women. PCOS is characterized by metabolic disorders, insulin resistance, anovulation, and hyperandrogenism. Its aromatic properties make BCP an important component of many products, despite its structural similarities to cannabinoids. In this study, BCP was studied for its impact on ovarian follicle quantity and quality in PCOS rats and for its impact on weight and ovarian size in these rats. A significant difference was found between the BCP-treated control group and the estradiol valerate-treated control group in terms of LH and FSH levels. A BCP treatment also elevates estradiol and progesterone levels, decreases testosterone, restores antioxidant enzyme activity, and reduces oxidative stress in ovarian tissue. Further, BCP treatment reverses the histomorphometric changes in ovarian structure seen in PCOS rats and increases the number of ovarian follicles. According to these studies, BCP might be effective in treating metabolic and reproductive problems related to PCOS.

KEYWORDS: Polycystic ovary syndrome (PCOS), Beta-caryophyllene (BCP), Reproductive disorders, Ovarian follicles, Antioxidant therapy.

INTRODUCTION

An endocrine disorder characterized by metabolic disturbances and related health problems, polycystic ovary syndrome (PCOS) is common among women during their reproductive years.[1] A person with PCOS often experiences irregular periods, signs of hyperandrogenism, and elevated LH: FSH levels. Anovulation and hyperandrogenism are caused by hyperinsulinemia, which results from insulin resistance.[2] There is now a greater appreciation for PCOS as a metabolic syndrome encompassing hyperinsulinemia, hyperlipidemia, diabetes mellitus, and possibly heart disease.[3] Other conventional symptoms include increased androgen levels, cosmetic concerns, anovulation, infertility, and obesity.[4] However, polycystic ovaries still pose a controversial diagnostic dilemma despite their prevalence. In recent years, there has been a growing emphasis on plant research worldwide, with accumulating evidence showcasing the vast potential of medicinal plants utilized in various traditional healing systems.[5] Pepper, clove, and cinnamon are all spicy spices due to the presence of BCP (bicyclic sesquiterpene lactone). BCP is classed as a phytocannabinoid and considered a "dietary cannabinoid" due to its structural and functional similarities with cannabinoids. Its aromatic properties make BCP useful in any number of products, including candies, confectioneries, chewing gum, toothpaste, beverages, pharmaceuticals, and cosmetics.[6] However, BCP exhibits low water solubility, making its bioavailability challenging. This may lead to its anti-inflammatory, antioxidant, neuroprotective, and anticancer activities. A potential solution, however, lies in liposomal formulations, which can be used to enhance the absorption of drugs and ensure desired biological results. CB2 receptors are predominantly responsible for modulating inflammatory pathways[7], while CB1 receptors exert their effects through cannabinoid type 1 (CB1) receptors. Among inflammatory diseases such as osteoporosis, osteoarthritis, colitis, and atherosclerosis, BCP has demonstrated anti-inflammatory activity against various inflammatory conditions.[8] This study aims to investigate the potential beneficial effects of BCP on the number and quality of ovarian follicles in PCOS. Additionally, the study will explore the impact of BCP treatment on body weight and ovarian weight in PCOS rats, shedding light on its potential therapeutic applications in managing this complex endocrine disorder.

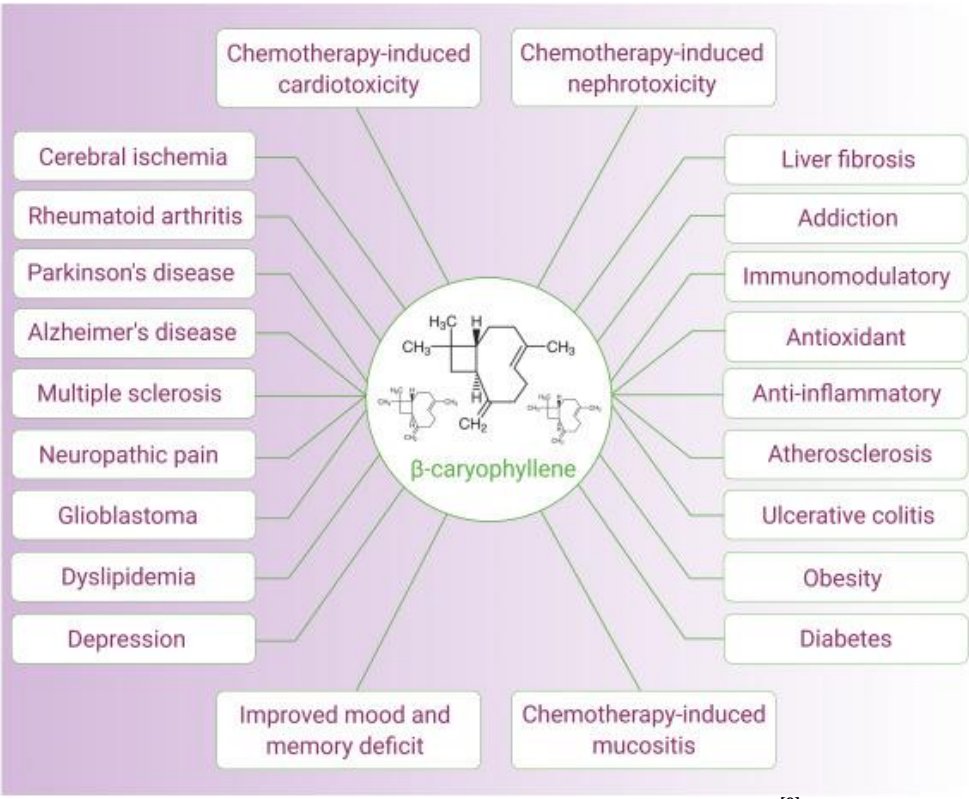


Figure 1: Beta-caryophyllene's Therapeutic potential.[9]

METHODOLOGY

Animal grouping

Rats were randomly divided into five groups, each containing six rats per cage. All animals in four groups received intramuscular injections of estradiol valerate at a dose of 4mg/kg dissolved in sesame oil, except for the normal control group. Thirty days after PCOS induction, rats in groups G4 and G5 were orally dosed by gavage for 15 days, while rats in the standard group received treatment for 5 days.

Table 1: Grouping of Animals.

Group of animals	Treatment protocol
G-I	Normal control
G-II	Disease control (PCOS)
G-III	Clomiphene citrate injection at 20 mg/kg body weight intra peritoneally, for 5 days.
G-IV	Treated with β- caryophyllene 150 mg/kg body weight, through orally, for 15 days.
G-V	Treated with β- caryophyllene 300 mg/kg body weight, through orally, for 15 days.

PCOS induction

Estradiol valerate (Bayer Zydus Pvt. Ltd.) dispersed in sesame oil was administered intramuscularly at a dose of 4 mg/kg to induce PCOS. The presence of PCOS was confirmed through a vaginal smear. Light microscopy was used to observe the vaginal smear daily, and the estrus cycle was confirmed for each rat.

Vagina smear

Vaginal smears were used to monitor estrous cyclicity. As a result of vaginal smear observation, two or three periods of regular estrus cycles were detected among the animals initially chosen for the study. In addition to irregular estrous cycles, persistent vaginal cornification (PVC) is an indication of ovarian follicle cysts.[10]

Histopathological determination

After the treatment period, all rats were weighed, anesthetized with ketamine/xylazine, and blood samples were collected via retro-orbital puncture. The serum obtained from the blood samples was used for hormonal assays, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, and testosterone. The ovaries were then excised, weighed, and homogenized for the estimation of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and malondialdehyde (MDA) levels. Histopathological examination was conducted on the ovaries. Additionally, the endometrial and myometrial thicknesses of the uterus were measured using Dino Capture 2.0 software.

Statistics

The results are presented as Mean ± SEM. Data were analyzed using ONE WAY ANOVA followed by Newman – Keul's multiple range test. Probability values less than ($p < 0.01$) were considered statistically significant.

RESULTS

The administration of estradiol valerate (EV) resulted in a significant increase in LH levels and a decrease in FSH levels in the toxic control group (G2) compared to the normal control group (G1) at $P < 0.001$. Consequently, the LH/FSH ratio was notably elevated in the toxic group, whereas both doses of β-caryophyllene (150mg/kg and 300mg/kg) led to a lower LH/FSH ratio and a significant decrease ($p < 0.001$) in LH levels accompanied by a rise in FSH levels compared to the toxic control group. Furthermore, there was a significant reduction in estradiol levels following EV injection after 30 days ($p < 0.001$), which was reversed by concurrent administration of β-caryophyllene for 15 days, resulting in a significant increase in estradiol levels ($p < 0.001$). Similarly, the standard group also exhibited a significant rise in estradiol levels. Additionally, treatment with β-caryophyllene at both doses (150mg/kg and 300mg/kg) along with EV significantly increased progesterone levels ($p < 0.001$) to near-normal values, similar to the standard group. Moreover, exposure to EV for 30 days led to a significant increase in testosterone levels ($p < 0.001$), which were also significantly elevated following treatment with β-caryophyllene at doses of 150mg/kg and 300mg/kg for 15 days, as well as after clomiphene treatment (table.2). Table 3 presents the alterations in SOD, CAT, GSH, and GPx activities in ovarian tissue among the toxic control and treatment groups. SOD, CAT, and GPx activities were significantly decreased in the PCOS groups compared to the normal control groups ($p < 0.001$). However, treatment with beta-caryophyllene resulted in a significant increase in SOD, CAT, and GPx levels compared to the induced PCOS rats ($p < 0.001$). Additionally, as indicated in Table 2, a significantly higher level of MDA was observed in the homogenate of PCOS groups compared to normal control groups ($p < 0.001$). Nevertheless, the level of MDA was significantly reduced in the treatment groups compared to the toxic control group ($p < 0.001$). In the PCOS group, there was a significant decrease in the numbers of preantral follicles, antral follicles, and corpus luteum compared to the control groups ($P < 0.001$). However, treatment control led to an increase in the number of follicles, although the corpus luteum count did not change. Other parameters returned to the normal range ($P < 0.001$) (table 4). Histomorphometric evaluation of ovaries revealed that the thickness of the granulosa and tunica albuginea increased, while the thickness of the theca layer decreased in the PCOS group compared to the control groups. Treatment with beta-caryophyllene altered these values toward normal levels ($P < 0.001$). There was no significant difference observed in the thickness of the endometrial and myometrial layers of the uterus between the groups.

Table 2: Effect β-caryophyllene on serum hormonal parameter in Estradiol valerate induced PCOS.

Groups	LH	FSH	Estradiol	Testosterone	Progesterone`
G-I	8.23±0.18	4.03±0.14	953.15±15.49	1.62±0.12	56.29±1.43
G-II	24.71±1.19**a	2.27±0.07**a	191.75±7.77**a	8.65±0.14**a	19.13±1.56**a
G-III	9.88±0.27**b	2.97±0.05**b	841.14±17.17**b	3.86±0.08**b	42.39±1.57**b
G-IV	13.71±0.39**b	3.56±0.06**b	706.92±16.50**b	5.67±0.14**b	31.59±1.38**b
G-V	11.29±0.51**b	3.28±0.06**b	752.15±16.86**b	4.62±0.14**b	38.16±0.88**b

All values expressed as means ± SEM; **a – values are significantly different from normal control (G1) at P<0.001; **b - values are significantly different from PCOS control (G2) at P<0.001.

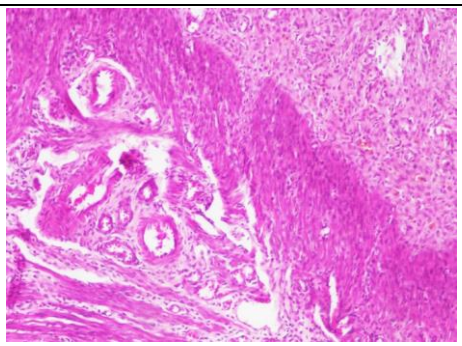
Table 3: Effect of β-caryophyllene on anti-oxidant enzymes.

Groups	SOD µ mol/g of tissue	Catalase µ mol/g of tissue	GPX µ mol/g of Tissue	MDA µ mol/g of tissue
G-I	4.40±0.08	18.65±0.18	8.48±0.26	0.5±0.03
G-II	2.34±0.07**a	5.65±0.18**a	3.28±0.18**a	0.8±0.034**a
G-III	3.65±0.09**b	17.56±0.30**b	7.38±0.21**b	0.59±0.01**b
G-IV	3.44±0.05**b	12.68±0.25**b	6.33±0.17**b	0.67±0.01*b
G-V	3.73±0.09**b	14.51±0.22**b	6.76±0.20**b	0.56±0.02**b

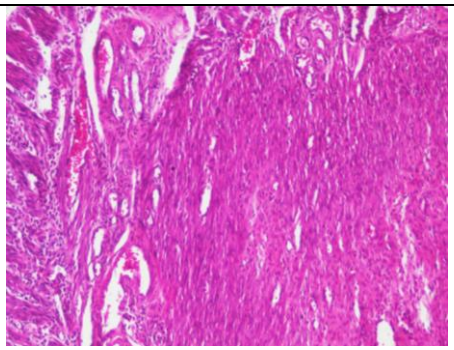
All values expressed as means ± SEM; **a – values are significantly different from normal control (G1) at P<0.001; *b – values are significantly different from PCOS control (G2) at P<0.01; **b - values are significantly different from PCOS control (G2) at P<0.001.

Table 4: Histomorphometric comparison of uterine tissue layer after treatment with clomiphene citrate in PCOS rats.

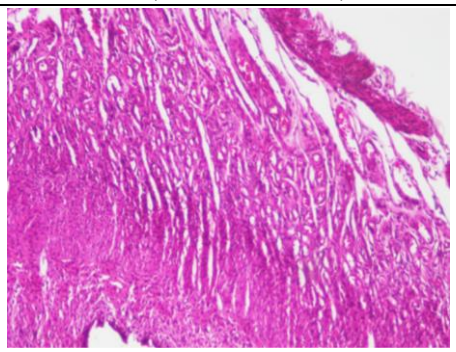
Groups	Endometrium thickness (µm)	Myometrium thickness (µm)
G-I	243.70±7.32	135.27±4.84
G-II	293.97±12.56	124.32±2.76
G-III	274.42±11.89	131.13±2.32
G-IV	291.95±8.85	125.75±2.78
G-V	287.21±7.98	128.38±1.51



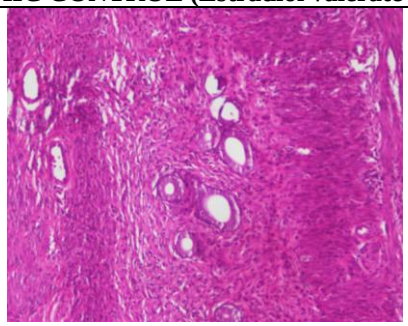
G1 (Normal Control)



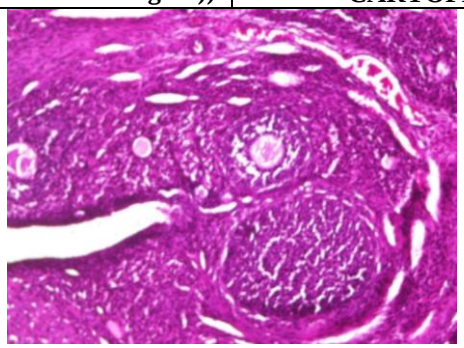
G2 (TOXIC CONTROL (Estradiol valerate4mg/kg))



G3 (STD CONTROL (Clomiphene Citrate 20mg/ml))



G4 (TREATMENT CONTROL (LOW DOSE OF β-CARYOPHYLLENE – 150 mg/kg))



G5 (TREATMENT CONTROL (HIGH DOSE OF β-CARYOPHYLLENE – 300mg/kg))

Figure. 2: Histopathological findings.

DISCUSSION

The study findings indicate that the administration of estradiol valerate (EV) induced significant hormonal imbalances characteristic of polycystic ovary syndrome (PCOS), including reductions in progesterone and estrogen levels, along with increased levels of luteinizing hormone (LH) and decreased follicle-stimulating hormone (FSH). These imbalances adversely affect follicular growth and ovulation, contributing to the pathogenesis of PCOS.[11] Beta-caryophyllene treatment mitigated these hormonal alterations, restoring FSH levels and reducing the LH/FSH ratio, thereby promoting follicular growth.[12] Additionally, beta-caryophyllene exhibited antioxidant properties, reducing oxidative stress markers such as malondialdehyde (MDA) levels. Oxidative stress is implicated in PCOS-related ovarian damage and follicular degeneration.[13] Treatment with beta-caryophyllene alleviated these histomorphological changes, reducing the number of cystic follicles and promoting the growth and development of follicles and corpus luteum.[14,15] These findings suggest that beta-caryophyllene may hold therapeutic potential in managing PCOS by addressing hormonal imbalances and oxidative stress, thereby improving ovarian function and follicular development.

CONCLUSION

PCOS is characterized by decreased antioxidant enzyme levels, cystic follicle formation, and disruption of follicular structure. Our results highlight the importance of oxidative stress in PCOS pathogenesis. Physical, biochemical, and histological changes associated with PCOS can however be reversed with beta-caryophyllene treatment. A study demonstrated a potent therapeutic effect of beta-caryophyllene against PCOS induced by ethinyl estradiol valerate by enhancing follicular growth and scavenging free radicals. Thus, beta-caryophyllene may offer a promising alternative therapeutic alternative for PCOS-related metabolic and reproductive disorders.

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