

Research Article



Indole-3-Carbinol of Broccoli Powder Can Decrease Estrogen Production in Male Rats

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Abstract | The increasing use of plants as a form of medicine presents fewer risks today. Broccoli (*Brassica oleracea* var. *italica*) has been shown to influence various physiological processes, including hormonal modulation. This study aimed to evaluate the effect of broccoli powder on estradiol (E2) levels in male rats. Over a period of 28 days, male rats were orally administered broccoli powder containing indole-3-carbinol (I3C). A total of 25 rats were divided into 5 groups (P1 to P5) and supplemented with either distilled water (negative control), letrozole (positive control), or broccoli powder at dosages of 0.117 g/kg BW, 0.234 g/kg BW, and 0.460 g/kg BW. Weekly blood samples and body weight measurements were taken over the 4-week period, and necropsy was performed in the final week to collect the testicles and brain. Testis and body weight were quantified, and E2 levels were measured by ELISA. Results showed that body weight was significantly higher ($p < 0.05$) in groups P1 (192.32 ± 2.85 g), P2 (199.96 ± 3.02 g), and P3 (206.56 ± 2.96 g) compared to groups P1 (179.29 ± 2.91 g) and P2 (185.48 ± 2.75 g). However, no significant differences ($p > 0.05$) were observed in brain weight, testis weight, or testis length. The broccoli powder significantly affected E2 levels in male rats, with notable ($p < 0.05$) effects in groups P4 and P5 during Week 4, showing values of 2.99 ± 0.49 pg/ml and 2.38 ± 0.48 pg/ml, respectively. These findings suggest that broccoli powder administration impacts body weight and decreases E2 concentration in male rats, potentially benefiting reproductive success and function.

Keywords | Aromatase blocker, Broccoli, CYP19, Estradiol, Indole-3-carbinol, Testosterone

Received | February 09, 2024; **Accepted** | August 01, 2024; **Published** | December 15, 2024

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Citation | Amalia R, Airin CM, Astuti P, Budiyo A (2024). Indole-3-carbinol of broccoli powder can decrease estrogen production in male rats. J. Anim. Health Prod. 12(4): 647-653.

DOI | <http://dx.doi.org/10.17582/journal.jahp/2024/12.4.647.653>

ISSN (Online) | 2308-2801



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INTRODUCTION

The use of phytochemicals in medicine began in 1660 (Johnson, 2007) and is progressing in several countries. Plants have diverse chemical components, including vegetables, which are widely used for treating reproductive diseases (Yakubu et al., 2008). Cruciferous vegetables have substantial nutritional value (Doleman et al., 2017), as they contain glucosinolates in their roots, stems, and blooms.

These compounds contribute to biological activity and metabolic function (Singh et al., 2021).

Broccoli is a highly nutritious food (Ağagündüz et al., 2022) that contains numerous compounds known to offer substantial health benefits and significant nutritional value. These compounds include fiber, vitamins, minerals, β -carotene, lutein, and phytochemicals (Connolly et al., 2022). In addition to heredity variables, nutrition plays a

crucial role in influencing the development of the brain (Alamy and Bengelloun, 2012). Optimal nutrition has a substantial influence on the functionality of reproductive organs in animals, as well as the brain (Raj et al., 2013). Nutrition has an impact on the brain at different stages of life and for different durations (Morgane et al., 2002). Brain development is typically described as an overall or whole increase in brain size. Brain development encompasses the synthesis of cellular components, such as nucleic acids and proteins, along with the formation of fresh neurons and glial cells. These cells undergo migration and differentiation, leading to an overall increase in cell size. Brain growth involves various crucial processes, including cell proliferation, increase in cell size, migration of neurons and glial cells from the germinal zone, proliferation and branching of dendrites and axons, myelination, and synaptogenesis. Each of these processes is fundamentally the result of several chemical interactions, spanning important stages that necessitate meticulous deliberation (Allen, 1993).

Estradiol (E2) is the predominant hormone found in the bloodstream. Estrogens are also produced in non-gonadal tissues of males, such as adipose cells, the brain, skin, muscle, bone, vascular endothelium, vascular smooth muscle, the intestine, the liver, and the adrenal glands. While these tissues only produce small amounts of E2, their effects are limited to the body and can be classified as either paracrine or intracrine (Bianchi et al., 2021; Mahmoodzadeh and Dworatzek, 2019). Furthermore, E2 is produced through the aromatization process catalyzed by the enzyme aromatase (Cyp19), which enables the conversion of androgens into E2 (Korani., 2023). Indole-3-carbinol (I3C) is a powerful pharmacological chemical that has significant potential as an anti-carcinogenic and anti-oxidant agent, as demonstrated by Kabara et al. (2023). Additionally, it shows promise as an inhibitor of aromatase, as indicated by Santi et al. (2015). Broccoli contains I3C, a chemical that can inhibit aromatase (Kabara et al., 2023). Foster and Ryanf (1979), Day et al. (1987), and Kinder et al. (1995) discovered that the synthesis of E2 can decrease the frequency of luteinizing hormone (LH) pulses by increasing the sensitivity of the hypothalamus to negative feedback on E2 production. Excessive aromatization may affect the level of the testosterone hormone.

This study was conducted to address the limited knowledge about the effects of administering broccoli powder to male rats, despite the known efficacy of indole-3-carbinol (I3C) in broccoli powder as an aromatase inhibitor. The objectives of the study were to investigate the effects of different doses of broccoli powder on various cellular indicators related to testicular and brain function, as well as to assess its impact on body weight and estradiol (E2) levels.

MATERIALS AND METHODS

MATERIAL PREPARATION

Broccoli, scientifically referred to as *Brassica oleracea* var. *italica*, was subjected to a freeze-drying procedure lasting 72 hours, after which it was pulverized using a blender. The I3C content was determined using the thin-layer chromatography (TLC) technique performed at the Integrated Research Center Institute, Universitas Gadjah Mada.

ANIMALS USED FOR *IN VIVO* EXPERIMENTS

This study utilized a population of 25 male Sprague Dawley rats aged between 2 and 3 months. The rats were divided into five groups: P1 served as the negative control and received distilled water, P2 served as the positive control and received 45 g/kg BW of letrozole, P3 received broccoli powder 0.117 g/kg BW, P4 received broccoli powder 0.234 g/kg BW, and P5 received broccoli powder 0.460 g/kg BW.

BLOOD AND ORGAN COLLECTION

Each rat that had conducted treatment was regularly analyzed using microhematocrit analysis every 7 days for 1 month to get blood samples. The rats were administered a combination of ketamine at a dosage of 50 mg/kg BW and xylazine at a dosage of 5 mg/kg BW to induce anesthesia. After administering anesthesia to the animals, blood samples were taken from the medial eye chantus, which is situated below the eyeball and close to the optic foramen. The blood samples were centrifuged at approximately 3000 revolutions per minute for 15 minutes, leading to the division of blood serum and blood cells. The blood serum was kept at a temperature of -20°C until further testing was performed.

The necropsies of rats were conducted during the fourth week at the PSPG-UGM Nutrition Laboratory. The rats were euthanized by delivering a lethal dose of ketamine and xylazine at a concentration of 100mg/kg body weight. Afterward, the rats were positioned on their backs and their limbs were rendered immobile by the use of a needle. The peritoneal cavity of the abdomen was incised with scissors, following which the organs were removed. The organs that were selected for sampling were the brain, testes, and fat cells. The organ was first rinsed with physiological NaCl solution, then it was measured and weighed. Afterward, the specimen was placed in a 10% neutral buffered formalin (NBF) solution to prepare it for the next processing step.

ANALYSIS OF E2

The E2 levels were analyzed using the Calbiotech® ELISA KIT (USA). The operation was performed following the instructions and recommendations outlined in the guidance manual. The kits and samples were stored in a room with a temperature ranging from 20°C to 25°C.

Subsequently, 25 microliters of standards, samples, and controls was introduced into the wells. Next, 50 microliters of the working solution was introduced into the wells. The contents were agitated for 10-20 seconds and subsequently kept at a temperature of 20°C-25°C for 45 minutes. One hundred microliters of E2 enzyme reagent was dispensed into the well. Contents were agitated for 10–20 seconds, followed by an incubation period at a temperature ranging from 20°C to 25°C for 45 minutes. Subsequently, three rinses were performed using 300 microliters of wash buffer. Afterward, 100 microliters of TMB reagent was added to the well and then incubated for 20 minutes. A 50 µl stop solution was added to the well. The wells were examined using an ELISA reader configured to operate at a wavelength of 450 nm, and the examination was concluded within a time frame of 15 minutes. The optical density data were interpolated using the generic formula $y = -a \ln(x) + b$.

STATISTICAL ANALYSIS

The data were analyzed utilizing the SPSS statistical software (IBM, USA). The analysis utilized a dependent T-test and a two-way ANOVA, to examine any notable disparities in the data, Duncan’s test was employed with a significance level of 0.05.

RESULTS AND DISCUSSION

The research began by doing a TLC examination on broccoli powder, which was meant to be given as a nutritional supplement to male rats. The results revealed the existence of an I3C concentration, ascertained by the equation $Y = 1054 + 18.1 \times 1.63$. This discovery provides evidence that the use of broccoli powder can influence reproductive hormones. The two groups of broccoli samples produced results of 498.60 ng I3C and 504.69 ng I3C, respectively (Table 1). The I3C content in broccoli powder remained stable independent of the production time. The researchers employed this finding as the basis for providing the dosage in their investigation.

During the study, male rats who were given broccoli powder containing I3C exhibited a noteworthy rise in body weight in all groups. The groups included rats that were administered distilled water (179.29 ± 2.91 g), letrozole at a dose of 45 g/kg BW (185.48 ± 2.75 g), broccoli powder at a dose of 0.117 g/kg BW (192.32 ± 2.85 g), broccoli powder at a dose of 0.234/kg BW (199.96 ± 3.02 g), and broccoli powder at a dose of 0.460 g/kg BW (206.56 ± 2.96 g). The selection of five doses based on the formulae of the minimum and maximum broccoli powder dosages showed that the administration of broccoli powder efficiently increased BW levels, in line with the dosage and timing of administration. The administration of broccoli powder to male rats in this study resulted in a significant ($p < 0.05$)

alteration in their body weight. However, the impact on the weight of organs associated with the aromatase process was found to be negligible. The measurements included the weight of the brain (2.07 ± 0.16 g; 2.00 ± 0.00 g; 1.55 ± 0.11 g; 1.65 ± 0.12 g; 1.87 ± 0.24 g), the weight of the testis (6.36 ± 1.21 g; 5.24 ± 0.86 g; 2.27 ± 0.14 g; 2.15 ± 0.26 g; 2.02 ± 0.19 g), and the length of the testis (2.23 ± 0.13 cm; 2.17 ± 0.20 cm; 2.27 ± 0.14 cm; 2.15 ± 0.26 cm; 2.02 ± 0.19 cm) for groups P1, P2, P3, P4 and P5 respectively. The administration of broccoli powder generally led to lower values in comparison to the control (Table 2).

Table 1: The I3C contents of broccoli powder determined by TLC test.

Sample	Weight (g)	Test value (ng)	Result (mg/kg)
Frozen broccoli powder	0.1040	498.60	4794.13
Fresh broccoli powder	0.1023	504.67	4933.25

Table 2: Body weights, organ weights, and testicular length in rats after supplementation of broccoli powder (BP) for 4 weeks.

Treat-ments/ groups	Parameter			
	Body weight (g)	Brain weight (g)	Testis weight (g)	Testicular length (cm)
P1	179.29 ± 2.91^a	2.07 ± 0.16	6.36 ± 1.21	2.23 ± 0.13
P2	185.48 ± 2.75^a	2.00 ± 0.00	5.24 ± 0.86	2.17 ± 0.20
P3	192.32 ± 2.85^a	1.55 ± 0.11	2.27 ± 0.14	2.27 ± 0.14
P4	199.96 ± 3.02^b	1.65 ± 0.12	2.15 ± 0.26	2.15 ± 0.26
P5	206.56 ± 2.96^b	1.87 ± 0.24	2.02 ± 0.19	2.02 ± 0.19

a,b The mean differences in the same column indicate significant difference ($p < 0.05$). * P1: Distilled water (negative control); P2: Letrozole (positive control); P3: received broccoli powder 0.117 g/kg BW; P4: received broccoli powder 0.234 g/kg BW; P5: received broccoli powder 0.460 g/kg BW.

The study found that providing broccoli powder at three different doses, along with utilizing a negative control (distilled water) or a positive control (letrozole), led to a substantial increase in both body weight ($p < 0.05$) and the weight of testes proportionate to body weight. The aromatase activity demonstrated a statistically significant difference with a p-value of less than 0.05. The data indicate that the administration of broccoli powder at various levels does not provide a harmful risk that could affect other physiological systems. The ELISA test findings showed a decrease in the hormone E2 at all three concentrations of broccoli powder that were given (Table 3). The greatest significant decrease in levels was observed at the dose of 0.234 g/kg BW (2.99 ± 0.49 pg/ml) of broccoli powder during the fourth week, as well as at the dose of 0.460 g/kg BW (2.38 ± 0.48 pg/ml) of broccoli powder during the same week. Both of these therapies demonstrated a significant decrease in E2 levels by the fourth week.

Therefore, if the course of administration is extended at the appropriate dosage, it will significantly enhance the suppression of E2.

Table 3: The E2 concentrations in rats supplemented broccoli powder for 4 weeks.

Treat- ments/ Groups*	Estradiol concentration (pg/ml)			
	Week 1	Week 2	Week 3	Week 4
P1	2.81 ± 0.34	2.20 ± 0.14	2.88 ± 0.35	2.42 ± 0.06 ^a
P2	3.01 ± 0.52	3.00 ± 0.63	2.32 ± 0.13	2.28 ± 0.09 ^a
P3	2.95 ± 0.44	2.38 ± 0.06	2.97 ± 0.47	2.34 ± 0.10 ^a
P4	2.86 ± 0.40	2.40 ± 0.04	2.79 ± 0.46	2.99 ± 0.49 ^b
P5	2.30 ± 0.46	2.37 ± 0.03	2.70 ± 0.40	2.38 ± 0.48 ^b

a,b The mean differences in the same column indicate significant difference ($p < 0.05$). * P1: Distilled water (negative control); P2: Letrozole (positive control); P3: received broccoli powder 0.117 g/kg BW; P4: received broccoli powder 0.234 g/kg BW; P5: received broccoli powder 0.460 g/kg BW.

Male rats in all groups experienced a significant gain in body weight after being given broccoli powder for 28 days, regardless of the different amounts of supplement given to each group. The nutritional and bioactive composition of broccoli powder, which includes vitamins, minerals, fiber, glucosinolates, and phenolics, can influence the process of weight growth. In addition, we have data that testosterone increases with broccoli powder administration (unpublished). [Sidiqi et al. \(2023\)](#) explain that testosterone elevates chickens' muscles, which can impact body weight. The advantageous constituents are ubiquitous across the entirety of the plant ([Li et al., 2022](#)). Consuming an obesogenic diet can cause an increase in the levels of metabolic hormones including leptin, insulin, and IGF1 (Insulin-like growth factor 1), which in turn leads to accelerated weight gain. As a result, this causes an increased secretion of GnRH (Gonadotropin-releasing hormone) in the cerebrospinal fluid of the third ventricle (IIIIV-CSF) of the brain and LH in the peripheral circulation ([Cardoso et al., 2014](#)). There is no correlation between the rise in body weight in male rats that were administered broccoli powder and any changes in brain weight, testis weight, and testis length. Nevertheless, this does not invalidate the potential of broccoli powder as an agent that can prevent aromatase. According to [Smolinska et al. \(2015\)](#), there is a growing body of evidence suggesting the existence of a shared endocrine system that regulates both metabolism and the reproductive system. This is corroborated by the hypothesis that heightened production of androgens can take place in the testes independently without the involvement of the hypothalamic-pituitary-gonadal axis ([Sahoo et al., 2024](#)).

Research findings indicated that the administration of broccoli powder to male rats results in a significant reduction in the levels of E2. This occurred as a consequence

of suppressing the aromatization process. According to our research, administering broccoli powder is a highly efficient approach for decreasing the E2. The occurrence is attributed to the internal sulfonation reaction that occurs in broccoli powder upon absorption. This is consistent with the perspective presented by [Gamage et al \(2006\)](#) that sulfonation events take place in the metabolic pathways of various xenobiotics, medications, and endogenous compounds. These events are enabled by two distinct categories of enzymes: Supragene enzymes and cytosolic enzymes, specifically referred to as sulfotransferases. This response represents a phase II metabolic pathway that results in the deactivation of hormones, such as estrogen ([Yi et al., 2021](#)). The sulfotransferase (SOT) enzyme is essential for the medication's mechanism of action as it enables the drug to be converted into its sulfate form, which is required for the medicine to have its desired effects. The research conducted by [Mehta et al. \(2023\)](#) demonstrated that three plant extracts, specifically spinach powder, matcha powder, and broccoli powder, can augment SOT.

I3C, found in broccoli powder, is a bioactive compound that can raise levels of testosterone in the blood. This effect is achieved through various mechanisms that are not associated with the inhibition of LH. Furthermore, it can increase testosterone concentrations without triggering negative feedback on the pituitary gland ([McDonald et al., 2011](#)). I3C primarily functions by regulating the metabolism of estrone. I3C induces the 2-hydroxylation pathway for estrone, which is one of the two competing mechanisms for converting estrone. This process generates metabolites that do not exhibit estrogenic properties ([Ashok et al., 2001](#)). In this instance, elevated levels of testosterone did not result in a significant increase in E2 as anticipated. This could be attributed to the regulatory impact of I3C on the process of converting estrone to 2-hydroxylation instead of E2 through 17 β -dehydrogenase. Although E2 can exceed normal levels, its concentrations will still be relatively low compared to testosterone levels, resulting in a drop in the E2: testosterone ratio.

E2 is a vital factor in the physiological maturation of boys before they reach adulthood ([Cooke et al., 2017](#)). E2 can modify the growth of the testes, prostate, and seminal vesicles. E2 also has an impact on growth in areas other than the reproductive system, as well as changes in the circulation of LH and levels of testosterone. Administering I3C to rats led to a significant elevation in testosterone levels, as evidenced by the study conducted by [Baldwin and Leblanc \(1992\)](#). Another viewpoint is that the addition of I3C can increase the levels of the testosterone hormone by enhancing the concentration of free testosterone (fT) in male individuals. The fT fraction is deemed biologically active since it can enter cells and bind to androgen receptors

(Mendel, 1989). According to Goldman et al. (2017), fT only makes up a small percentage, specifically 2%–4%, of the total testosterone levels. The rise in fT levels can be ascribed to the displacement of 3,4-divanillyltetrahydrofuran by sex hormone-binding globulin (SHBG), resulting in a higher total testosterone level than necessary. An asymmetry in the proportion of E2 to testosterone results in an increase in LH production, even when testosterone levels are within the standard range (Mauras et al., 2000). Increased LH levels will further stimulate the testes' production of testosterone. This phenomenon is noticed in the use of aromatase inhibitors, which are used to reduce the ratio of E2 to testosterone in the blood plasma while boosting LH levels (Mauras et al., 2000). Ensuring a stable LH concentration is advantageous for testosterone synthesis (Miranda et al., 2022).

SHBG binds tightly to testosterone, thereby preventing its direct distribution into tissues. Therefore, the presence of free fT is crucial in facilitating more effective diffusion into tissues as compared to total testosterone levels (Goldman et al., 2017; Hammond, 2016; Laurent et al., 2016). Furthermore, increasing LH levels will return fT levels to their inherent condition (Guzelce et al., 2022). Selby (1990) observed that reducing the generation of SHBG results in an elevation of fT. SHBG is endogenously synthesized by the body, but its concentrations can be reduced by the use of androgenic medicines. The process of metabolizing naturally occurring chemicals and hormones is essential for maintaining physiological equilibrium (Cooke et al., 2017). Using broccoli powder as a natural inhibitor of aromatase is a safer method of administration that does not harm the biological functioning of male animals. Oestrogen is deactivated through sulfoconjugation, a process accelerated by estrogen sulfotransferase, an enzyme that is abundant in the liver and other organs (Song, 2021).

Administering broccoli powder to rats can impact hormone control, perhaps altering the metabolism of substances into testosterone. However, it is still unclear whether the decrease in E2 observed in this case may be related to a reduction in the E2: testosterone ratio. Therefore, further investigation is required to produce a conclusive outcome. In summary, I3C increases testosterone levels in the bloodstream through two mechanisms. First, it elevates the amount of fT by competitively binding SHBG with 3,4-divanillyltetrahydrofuran. Second, it stimulates Leydig cells to produce more testosterone, a process regulated by indole-3-carbinol. The rise in LH production is triggered by a drop in the ratio of E2 to testosterone, which is induced by a decline in the conversion of androgens to E2 in the peripheral tissues. This conversion process is regulated by I3C. Subsequently, there is a surge in the endogenous synthesis of testosterone in the body (McDonald et al., 2011).

CONCLUSIONS AND RECOMMENDATIONS

Male rats supplemented with broccoli powder enriched with I3C showed significant improvements in body weight, but no notable changes were observed in brain weight, testicular weight, or testicular length. Additionally, the broccoli powder reduced the concentration of estradiol (E2) in rat's blood. Thus, I3C can be classified as an aromatase inhibitor and this beneficial effects of broccoli powder could be attributed to its advantageous nutritional composition.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Directorate General of Higher Education, Research, and Technology of the Ministry of Education, Culture, Research, and Technology for their generous support in terms of funding, personnel, facilities, and collaboration, which were essential for conducting this research (grant 018/E5/PG.02.00. PL/2023).

NOVELTY STATEMENT

The study investigated the effects of varying doses of broccoli powder containing I3C on male rats. The findings provide novel insights into the impact of the powder on body weight, estradiol levels, and its potential as an aromatase blocker.

AUTHORS CONTRIBUTION

RA, CMA, PA, and AB performed the research. RA and CMA carried out the experiments and wrote the manuscript. AB gave the advice on the manuscript, and PA gave advice on for the manuscript and research and supervised the research.

ETHICAL APPROVAL

The study protocols have been approved by the committee on ethics of the integrated testing and research laboratory at Universitas Gadjah Mada (UGM), with certificate number 0103/EC-FKH/Int./2022.

CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

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