



Black tea extract ameliorates indomethacin induced changes in testicular histopathology of albino rats

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ABSTRACT

Context: Reactive oxygen species and lipid peroxidation play a role in the pathogenesis induced by the non-steroidal anti-inflammatory drug indomethacin. **Aims:** This study was aimed to assess the simultaneous effect of black tea extract (BTE) on indomethacin induced alteration in testicular pathology. **Materials and Methods:** Adult rats were divided into four groups ($n = 6/\text{group}$); Group I (control); Group II (indomethacin, 5 mg/kg b.wt.; i.p.); Group III (BTE, 2.5 g tea leaf/dL of water, i.e., 2.5% of aqueous BTE, orally) and Group IV (indomethacin + BTE). Serum vitamin E, sperm count, motility, and histopathology of testes were evaluated. **Statistical Analysis:** Statistical comparisons were performed using the one-way ANOVA, followed by *post-hoc t*-test. **Results:** Indomethacin induced rats showed a significant decrease in testicular weight, sperm count, sperm motility, and serum vitamin E concentrations. Histopathology of the testes revealed tortuous seminiferous tubules, loss of spermatogenesis process, marked decrease in the number of interstitial cells of Leydig and been replaced by many foci of congestion, edema, necrosis, and interstitial fibrosis. Rats simultaneously treated with BTE and indomethacin showed improvement of testicular weight, sperm count, sperm motility, and serum vitamin E concentrations when compared to indomethacin alone treated rats. Similarly, histopathology of rats treated simultaneously with indomethacin and BTE showed near normal testicular architecture when compared to indomethacin alone treated rats. **Conclusion:** The result suggests that BTEs might have potential beneficial effect to combat against indomethacin induced testicular damage in rats.

Key words: Black tea extract, indomethacin, testicular damage, vitamin E

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used in medication in the world because of its efficacy to reduce pain and inflammation.¹ Its efficacy has been documented in a number of clinical disorders, including osteoarthritis, rheumatoid, arthritis, ankylosing spondylitis, gout, dysmenorrhea dental pain and headache.²

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Indomethacin is a NSAID drug and associated with several side-effect like nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache, drowsiness, and gastric ulcers. There are some reports on indomethacin that it induces detrimental effects on reduction in seminal volume, sperm count, and motility.³ Indomethacin leads to mitochondrial oxidative stress associated with the generation of intra-mitochondrial reactive oxygen species (ROS), which induces imbalance of oxidants and antioxidants status in living system.⁴ ROS and lipid peroxidation play a role in the testicular pathogenesis induced by the NSAID like indomethacin.⁵

Black tea extracts (BTE) acts as an effective suppresser of ROS in animals as it is a good source of antioxidants especially flavonoids. Moderate intake of black tea improves the levels of independent risk factors of cardiovascular disease and antioxidant defenses in plasma.⁶

The present study was aimed to assess the protective role of BTE on indomethacin induced alteration of testicular pathogenesis in albino rats.

MATERIALS AND METHODS

Wister strain of albino male rats aged 50-60 days and 160 ± 10 g body weight was used in this study. All animals were fed with water and food *ad libitum*. Experiments were carried out after obtaining Institutional Animal Ethical Committee approval. Animal breeding and maintenance were done in accordance with guidelines of Government of India for use of laboratory animals.⁷

Preparation of 2.5% aqueous BTE

The black tea (*Camellia sinensis*) extract was prepared from cut, tear and crush broken orange pickoe grade black clonal tea. It was processed and supplied by Tocklai Experimental Station, Jorhat, Assam. A fresh 2.5% aqueous BTE was prepared every day following the method of Wei *et al.*⁸ Twenty-five gram of black tea was added to 500 mL of boiling water and was steeped for 15 min. The infusion will be cooled to room temperature and then filtered. The tea leaves were extracted a second time with 500 mL of boiling water and filtered, and the two filtrates were combined to obtain a 2.5% aqueous BTE (2.5 g of tea leaf/100 mL water). The resulting clear solution is similar to tea brews consumed by humans.⁹

The Wister strain male albino rat (150-170 g) were chosen, and animals were divided into four groups, i.e., Group I: Control; Group II: Indomethacin (5 mg/kg b.wt.; i.p. for

21 days);¹⁰ Group III (BTE dose of 2.5 g tea leaf/dl of water that is 2.5% of aqueous BTE also for 21 days orally) and Group IV (both indomethacin and BTE simultaneously for 21 days). All the animals were sacrificed at the end of the last dose after an overnight fast. All the experimental procedures followed were performed in accordance with the approval of the Institutional Animal Ethics Committee (1169/ac/08/CPCSEA) under strict compliance of Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines for the experimental studies.

Gravimetry

The body weights of all animals were recorded on the 1st day of the indomethacin treatment and the day of sacrifice. The testicular weight was determined after dissecting out, and washing in ice-cold saline in a single-pan balance (ATCO. M. No. D2RS02-W).

Animal sacrifice and serum collection

At the end of the last dose, the animals were sacrificed after overnight fasting by decapitation between 09.00 AM and 11.30 AM to avert the circadian influences on the animals.¹¹ Blood was collected in centrifuge tubes, kept at room temperature for about 2 h and centrifuged at 1500 ×g for 15 min to collect serum.

Vitamin E assay

Serum was used for the estimations of α-tocopherol concentration by the modified method of Jargar *et al.*¹²

Determination of sperm count and motility

Both the sperm count and motility was determined by the method of Makler.¹³ Briefly; a sperm count was performed on sperm samples collected from the cauda epididymis. The cauda portion was separated and placed in 0.25 M sucrose solution. The epididymal tubes were punctured with a fine hypodermic needle and spermatozoa were extruded by squeezing. Sperm heads within a 10² area were counted in the same manner as blood cells counted in a hemocytometer; their number represents their concentration in millions/mL.

Histopathological studies

After sacrifice, testis of the experimental rats were dissected out (of one side) and stored in Bouin's fixative solution and processed. Fixed tissues were cut and stained with hematoxylin and eosin accordingly.¹⁴ The sections were examined under a light microscope, and photomicrographs

were taken by using USB MIPS (NO. 70.0343) with a connected personal computer.

Statistical analysis

Data were expressed as mean \pm standard deviation of the mean. Statistical comparisons were performed by one-way ANOVA, followed by *post-hoc t*-test, and the values were considered as statistically significant when $P < 0.05$.

RESULTS

Gravimetry

Table 1 shows the lowest percentage of body weight gain in case of Group II (indomethacin) rats. A significant improvement of percentage of body weight gain was observed in the indomethacin treated rats which were supplemented with BTE. Group III (only BTE) rats did not show any significant changes as compared to Group I rats. Similarly, significant decreased of testicular weight were noticed in the case of Group II (indomethacin) rats, but in case of Group IV (BTE + indomethacin) a remarkable improvement in weight of testis were also observed.

Vitamin E Assay

From Table 2, it may be observed that indomethacin significantly decreases serum vitamin E levels (Group II). However, supplementation of BTE on indomethacin treated rats (Group IV) showed an improvement of the level of serum vitamin E concentrations (Figure 1).

Sperm count and motility

Table 2 shows indomethacin (Group II) induced a significant decrease in the sperm count and motility of the rat in comparison with their control (Group I). Simultaneous supplementation of BTE with indomethacin in Group IV rats, significantly improved sperm count as well as sperm motility when it compared with Group II rats.

Histopathological studies

The histological structure of normal untreated rat testis (Group I) showed normal architecture (Figure 2a and b). In Group II (Indomethacin) showed majority of the

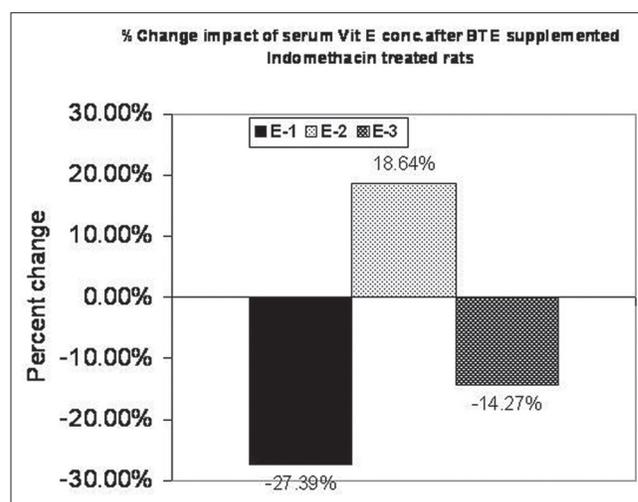


Figure 1: Percentage change of effect of black tea extract on serum vitamin E concentration in indomethacin treated rats. E-1, Group I versus Group II; E-2, Group I versus Group III; E-3, Group I versus Group IV

Table 1: Effect of BTE (2.5 g tea leaf/dl of water; oral) on gravimetric parameters in male albino rats after indomethacin (NSAID) treatment (5 mg/kg b. wt.; i.p.)

Gravimetry (Grams)	Group I	Group II	Group III	Group IV
Initial body weight	173.50 \pm 12.02 ^a	168.50 \pm 4.95 ^a	186.00 \pm 5.66 ^a	187.50 \pm 10.61 ^a
Final body weight	242.50 \pm 10.61 ^a	192.50 \pm 3.54 ^b	254.50 \pm 14.85 ^a	242.00 \pm 5.66 ^a
% of body weight gain	39.89 \pm 3.58 ^a	14.26 \pm 1.26 ^b	36.77 \pm 3.83 ^a	29.19 \pm 4.29 ^c
Testes	1.95 \pm 0.1224 ^a	1.233 \pm 0.1505 ^b	2.00 \pm 0.6324 ^a	1.5333 \pm 0.1966 ^c

Group I: Control, Group II: Indomethacin treated, Group III: BTE treated, Group IV: BTE + Indomethacin treated. Each value is mean \pm SEM of six observations in each group. In each row, values with different superscripts (a, b, c, d) were significantly different from each other ($P < 0.05$). *Post-hoc t*-test analysis was used to test for differences among the means when ANOVA indicated a significant $P < 0.05$. ANOVA: Analysis of variance, BTE: Black tea extract, NSAID: Non-steroidal anti-inflammatory drug

Table 2: Effect of BTE (2.5 g tea leaf/dl of water; oral) and sperm count, motility and biochemical parameter in male albino rats after indomethacin (NSAID) treatment (5 mg/kg b. wt. i.p.)

Test	Group I	Group II	Group III	Group IV
Vitamin E (μ g/ml)	15.55 \pm 0.942 ^a	11.29 \pm 0.47 ^b	18.45 \pm 0.47 ^c	13.33 \pm 0.47 ^d
Sperm count (millions/ml)	53.16 \pm 2.23 ^a	40.83 \pm 1.83 ^b	56.17 \pm 1.83 ^a	45.66 \pm 1.63 ^c
Sperm motility (%)	79.8333 \pm 1.1690 ^a	61.1666 \pm 4.4907 ^b	79.8333 \pm 0.7527 ^a	71.8333 \pm 2.4832 ^c

Group I: Control, Group II: Indomethacin treated, Group III: BTE treated, Group IV: BTE+Indomethacin treated. Each value is mean \pm SEM of six observations in each group. In each row, values with different superscripts (a, b, c, d) were significantly different from each other ($P < 0.05$). *Post-hoc t*-test analysis was used to test for differences among the means when ANOVA indicated a significant $P < 0.05$. ANOVA: Analysis of variance, BTE: Black tea extract, NSAID: Non-steroidal anti-inflammatory drug

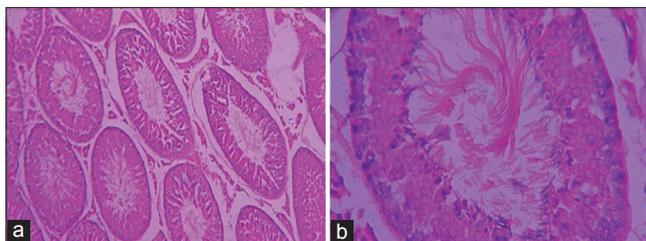


Figure 2: (a) Testis section of normal control Group I ($\times 10$), (b) testis section of normal control Group I rats ($\times 45$)

seminiferous tubules appear to be tortuous, marked thickening of tubular basement membrane and germ cell layer, with the loss of spermatogenesis up to 75%. The approximate percentage of spermatogenesis was counted on the basis of concentrations of matured spermatozoa available in the lumen of the seminiferous tubules. Lumen completely filled with sperms was considered as 100%. The fibro vascular stroma present between the seminiferous tubules; showed marked decrease in a number of interstitial cells of Leydig and these were replaced by many foci of congestion, edema, necrosis and interstitial fibrosis (Figure 3a and b). In Group III (BTE) showed normal architecture of histology of testis with spermatocytes, and normal interstitial cells of Leydig (Figure 4a and b). In Group IV (Indomethacin + BTE) showed a normal testicular parenchymal architecture with seminiferous tubule, and there is mild thickening of tubular basement membrane along with that few foci of loss of spermatogenesis up to 50% and increase in germ cell layer, with foci of interstitial congestion and edema (Figure 5a and b).

DISCUSSION

Analgesic and anti-inflammatory compounds have been associated with congenital cryptorchidism in humans, the best-known risk factor for low-semen quality and testicular germ cell cancer.¹⁵

Table 1 shows the lowest percentage of body weight gain in case of Group II (indomethacin) rats. It indicates that probably indomethacin interfere metabolic pattern of experimental animals. Another possible reason for the least percentage of body weight gain in case of indomethacin treated rats may be due to poor gastro intestinal functions with less absorption of nutrients. The reduction of testicular weight after indomethacin treatment may be also due reduction of protein synthesis or hormonal interferences in testes.¹⁶ Estimation of serum vitamin E concentration provides understanding of the circulatory antioxidant status, which facilitates to control the damage to the body from oxidative free radicals. It has been recognized since 1940s that vitamin E

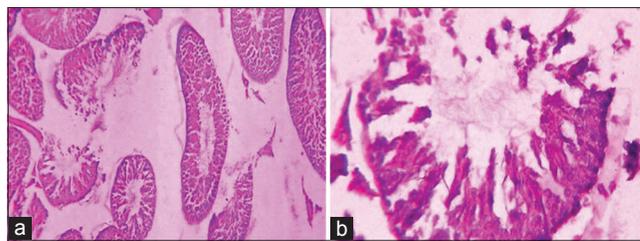


Figure 3: (a) Testis section of indomethacin induced Group II rat ($\times 10$), (b) testis section of indomethacin induced Group II rat ($\times 45$)

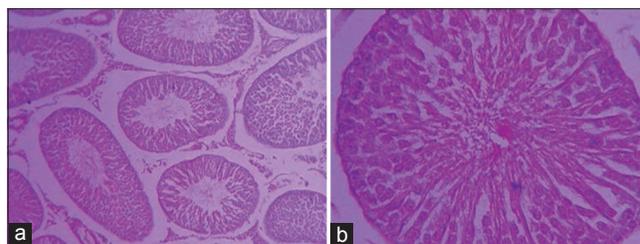


Figure 4: (a) Testis section of black tea extract (BTE) Group III rat ($\times 10$), (b) testis section of BTE Group III rat ($\times 45$)

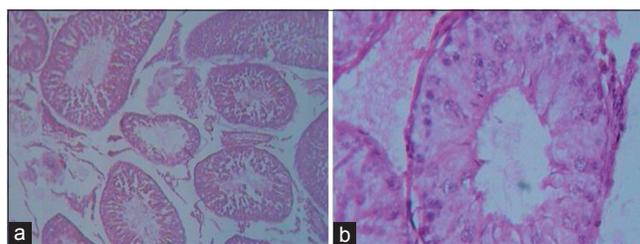


Figure 5: (a) Testis section of black tea extract (BTE) + indomethacin Group IV rat ($\times 10$), (b) testis section of BTE + indomethacin Group IV rat ($\times 45$)

is a powerful lipophilic antioxidant that is absolutely vital for the maintenance of mammalian spermatogenesis.¹⁷ In our study, low-serum vitamin E concentration in indomethacin treated rats clearly reflect the adverse effect of indomethacin on antioxidant and prooxidant balance in the blood.¹⁸ This critical balance between antioxidation/prooxidation was restored once again after simultaneous treatment of BTEs in the experimental Group IV. Tea flavonoids are potent antioxidants that are absorbed from the gut after consumption. Tea consumption consistently leads to a significant increase in the antioxidant capacity of the blood. Beneficial effects of increased antioxidant capacity in the body may be the reduction of oxidative damage to important biomolecules.¹⁹ However, the quality of the studies now available is insufficient to draw firm conclusions. In the present study the significant improvement in vitamin E concentration, sperm count and sperm motility and testicular architecture in Group IV rats treated concomitantly with BTE and indomethacin agrees with reports that BTE can be protective against the testicular abnormalities in rats.

At the level of the testes, indomethacin induced oxidative stress which may be capable of disrupting the steroidogenic capacity of Leydig cells as well as the capacity of the germinal epithelium to differentiate normal spermatozoa.^{20,21} A wide variety of different xenobiotics have also been shown to induce oxidative stress in the testes in concert with the suppression of antioxidant mechanisms. Oxidative DNA damage in the male germ line in association with a 32% reduction in the vitamin E content of the seminal plasma has already been reported.²²

In the present study, indomethacin induced decrease sperm count and motility suggests the destruction of the testicular germ cells by membrane damage or macromolecular degeneration. The present study also demonstrated that supplementation of BTE can have positive roles to improve sperm quality via protecting testicular cell membrane and mitochondria.

The results of our histopathological studies indicate that indomethacin probably crossed blood testes barriers and caused degeneration of seminiferous tubules, focal necrosis and decrease spermatogenesis. The change in cellular integrity could be due to the oxidative stress developed by the indomethacin-induced generation of ROS. A significant improvement of testicular architecture in BTEs supplemented indomethacin treated rats clearly indicates ameliorating effects of BTEs.

Hence, it may be concluded that testes are vulnerable to indomethacin but BTEs probably through its antioxidant properties combat against indomethacin induced testicular damage.

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