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The Original
DOI: https://doi.org/10.4238/vcmgqf62

THE EFFECT OF CINNAMON EXTRACT ON HORMONAL PARAMETERS IN METHOTREXATE INDUCED INFERTILITY IN FEMALE ALBINO RATS

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ABSTRACT

Objectives: To study the effect of cinnamon Extract on serum FSH, LH and AMH level in Methotrexate-induced infertility in albino rat.

Material & Methods: Prospective experimental animal study was done on 27 healthy female adult albino rats of 810 weeks, weighing 120-180gm, from the animal house of Baqai Medical University during February 2025 till August 2025. The rats were randomly divided into three groups: A, B and C, each group consisting of 9 animals. The animals from each group were kept in separate cages. Each group further divided into three sub groups, each subgroup contains 3 animals. Group-A (Control group) all subgroups A1, A2 and A3 was given normal saline intraperitoneally daily. Group-B (Methotrexate-induced group) all subgroups B1, B2 and B3 was given Methotrexate 2.5mg/kg intraperitoneally daily. Group C (Methotrexate plus Cinnamon-protected group) all subgroups C1, C2 and C3 was given Methotrexate 2.5mg/kg intraperitoneally daily plus Cinnamon Extract l00mg/kg intraperitoneally daily. Rats will be sacrificed at the end of their respective period of time i.e. 2nd, 4th and 6th weeks, blood was taken from intra cardiac puncture and analyzed through ELISA.

Results: All sub-groups within the methotrexate- induced group exhibited a marked reduction in body weight as compared with control and cinnamon-protected group. FSH and LH revealed statistically significant differences, as indicated by the p-value of <0.01. FSH and LH is significantly increased with methotrexate-induced group as compared with cinnamon-protected group. The methotrexate-induced group exhibited substantially lower AMH levels across all subgroups, while the cinnamon-protected group demonstrated AMH levels slightly lower than the control group, suggesting that the protective intervention effectively preserved ovarian reserve and reduced the decline observed in the methotrexate-induced group.

Conclusion: Cinnamon Extract could prevent the hormonal imbalance in Methotrexate induced infertility in albino rats.

KEYWORDS: Methotrexate, Cinnamon Extract, Anti-Mullerian Hormone (AMH), Follicle Stimulation Hormone, Luteinizing Hormone.

INTRODUCTION

Infertility is the inability to conceive after 12 months or more of consistent, unprotected sexual activity, a disorder of the female reproductive system[1]. Female infertility has a major effect on women's reproductive health all over the world. More than 110 million women were suffered in 2021, approximately 76.11% rise since 1990, according to recent studies showing a rising prevalence[2].

A variety of disorders affecting the ovaries, uterus, fallopian tubes, and endocrine system, can result in infertility in females. There are several causes of infertility, such as hormonal abnormalities, structural problems and drug toxicity

like steroids, antidepressants, antimetabolites medicines[3,4]. The drugs may cause problems with menstruation and ovulation, which may make conception difficult [5].

Antimetabolites are the most widely used and potent class of anticancer drugs. Since antimetabolites are the first logically created anticancer treatments that target RNA and DNA, they could be considered the first generation of targeted medications. Antimetabolites typically hinder cell division and the machinery involved in DNA replication[6,7]. Methotrexate, cyclophosphamide, and thioguanine were initially used to treat a variety of cancers, including leukemia and malignancies of the breast, ovaries, and gastrointestinal tract[8,9].

The cytotoxic drug methotrexate mainly functions as an antifolate by blocking important enzymes involved in the metabolism of folate, which is essential for cellular growth and operation. Reproductive results may be impacted by the action of methotrexate, which can have negative effects on trophoblast function and oocyte quality in ovary in the context of female infertility[10].

Several investigations have shown that methotrexate has a considerable negative impact on ovarian function and oocyte quality[9]. The effects of methotrexate on the ovaries can be divided based on tissue health and hormone levels. Hormonal effects of methotrexate therapy has been linked to altered levels of anti-müllerian hormone, a crucial indication of ovarian reserve[11]. Anti-müllerian hormone is a key determinant of female reproductive health and is currently recognized as one of the most accurate biomarkers for multiple applications within reproductive medicine[12]. It is important to carefully assess the Methotrexate dosage because increasing the dose could lower the ovary's reserve[13]. Moreover, the follicle-stimulating hormone, which helps in oogenesis, was indirectly affected by the amount of methotrexate used. Higher doses of methotrexate caused more damage to the ovaries than lower doses[14]. Cinnamon, scientifically known as Cinnamomum Zeylanicum Nees, is an aromatic and pleasant plant. Cinnamon possesses antispasmodic, carminative, anti-diarrheal, antibacterial, anti-parasitic, and cooling actions[15]. Cinnamon also helps reduce apoptosis and oxidative stress. Cinnamon oil reduces lipid peroxidation and enhances antioxidant enzyme activity, cinnamon oil significantly lowers oxidative stress markers in a various tissues, including the uterus and reproductive organs[16].

Cinnamon has been shown to increase the levels of luteinizing hormone, follicle stimulating hormone and antimullerian hormone by influencing the hypothalamic-pituitary-ovarian axis[17].

This study was aimed to observe the effects of cinnamon extract on imbalance of FSH, LH and AMH levels in methotrexate induced infertility in female rats. This experiment will provide a better management plan for the hormonal therapy in infertility subjects.

MATERIALS AND METHOD Study Design

The Prospective experimental study was conducted at the Department of Anatomy, Animal House, Fatima Hospital, Baqai Medical University, Karachi, for seven weeks, after the approval of the ethical committee (Ref: BMU-IREB/122024/006, Date 20/12/2024). Twenty seven healthy female adult albino rats of 8-10 weeks weighing 120-180 gm. were recruited for this study. The animals were housed in a clean, well-ventilated space with appropriate lightning, comfortable temperature, and good hygiene. The rats were fed rat chow as a regular balanced diet. Food and water were supplied ad libitum. They were kept under these conditions for one week to acclimatize them before starting the experiment. Animals were weighed before the start and on completion of their respective duration of treatment. One kilogram of cinnamon sticks was bought from the market and ground into a powder for the cinnamon extract. The soxhlet extraction method was used, which entailed filling a soxhlet machine with 200 milliliters of ethanol and water for every ten grams of cinnamon powder. A Rota vapor machine was then used to separate the solvent from the extract. This procedure was done in ICCBS (International Center for Chemical and Biological Sciences) in Karachi University.

Grouping

The animals were randomly divided into three groups: A, B and C each group consisting of 9 animals. The animals from each group were kept in separate cages.

Group-A was served as control, group-B was given Methotrexate & group-C was given Methotrexate plus Cinnamon Extract. Each group further divided into three sub groups according to the period of treatment, 2nd, 4th and 6th weeks. Each subgroup contains 3 animals.

Group A: (Control group): All subgroups A1, A2 and A3 was given normal saline intraperitoneally daily and was sacrificed at the end of their respective period of time i.e. 2nd, 4th and 6th week.

Group B: (Methotrexate-induced group): All subgroups B1, B2 and B3 was given Methotrexate 2.5mg/kg intraperitoneally daily and were sacrificed at the end of their respective period of time i.e. 2nd, 4th and 6th week[18].

Group C: (Methotrexate plus Cinnamon-Protected group): All subgroups C1, C2 and C3 was received Methotrexate 2.5mg/kg intraperitoneally daily plus Cinnamon Extract 100mg/kg intraperitoneally daily. Rats were sacrificed at the end of their respective period of time i.e. 2nd, 4th and 6th weeks[19].

Dissection and Tissue Sampling

The rats in groups A, B, and C were weighed and observations were noted at the conclusion of the experimental exposure. After a heart puncture, the blood samples were collected into vacutainers, kept in the refrigerator until they were centrifuged (PLC-05) for 20 minutes at 3000 revolutions per minute, and then kept in a freezer set at -20 degrees Celsius. Rat-specific kits (ELISA) were used to measure serum levels of LH, FSH, and Anti-Mullerian Hormone (AMH) after the serum was extracted from the blood sample[20].

Statistical Analysis

The data were entered and analyzed using SPSS 22. Mean and standard deviation for numeric. One way ANOVA and Post-Hoc Tuckey Test will be applied to assess the difference of mean between the studied groups. P value < 0.05 will be considered statistically significant.

RESULTS Body Weight

In the present study, the pre- and post-experimental body weights of animals were compared across the control group, methotrexate-induced group and cinnamon-protected group to evaluate the impact of the experimental interventions. Animals in the control group showed a consistent increase in body weight from baseline to the end of the experiment, reflecting normal growth. In contrast, all sub-groups within the methotrexate-induced group exhibited a marked reduction in body weight following treatment. Animals in the cinnamon-protected group also demonstrated a decrease in body weight after treatment; however, the reduction was noticeably less severe than that observed in the methotrexate-induced group.

Absolute Weight of the Ovary

The comparison of absolute ovarian weight among the control, methotrexate-induced, and cinnamon-protected groups. In the control group, ovarian weight ranged from 43 ± 1.73 to 52 ± 2, reflecting normal conditions and healthy ovarian development. In contrast, animals in the methotrexate-induced group showed a marked and progressive reduction in ovarian weight across sub-groups, with values decreasing from 38.67 ± 1.15 in subgroup 2 week to as low as 31.33 ± 2.31 in subgroup 6 week. This significant decline indicates that the methotrexate-induced group condition adversely affected ovarian mass, likely due to structural degeneration, follicular loss, or impaired tissue integrity. The cinnamonprotected group displayed ovarian weights that were intermediate between the control and methotrexate-induced groups. Sub-groups 4 week and 6 week (41.33 ± 1.15 and 41.67 ± 3.21) showed values close to the control group, suggesting a strong protective effect, while subgroup 2 week (38.33 ± 1.53) remained slightly lower but still higher than the corresponding methotrexate-induced sub-group. The highly significant p-value (0.001) confirms meaningful statistical differences among the groups.

Relative Ovarian Weight

The comparison of relative weight among the control, methotrexate-induced and cinnamon- protected groups. In the control group, relative weight values ranged from 23.41 ± 1.94 to 27.04 ± 1.25, representing normal conditions. In the methotrexate-induced group, relative weight showed a downward trend, decreasing from 24.17 ± 0.74 in subgroup 2 week to 21.85 ± 1.26 in subgroup 6 week. This reduction indicates that the methotrexate-induced condition negatively affected the weight of animals. In contrast, the cinnamon-protected group showed relative weight values closely aligned with the control group. Sub-groups 2week, 4week, and 6 week (23.28 ± 0.77, 24.41 ± 0.78, and 24.5 ± 2.41, respectively) demonstrated stable weights, suggesting that the cinnamon-protected treatment helped maintain normal balance and prevented the decline observed in the methotrexate-induced group. The p-value of 0.017 confirms that the differences among the groups are statistically significant. Overall, the results indicate that the methotrexateinduced condition leads to a measurable reduction in relative weight, while the cinnamon-protected treatment effectively preserves stability and prevents methotrexate-induced deterioration.

Serum Analysis of Hormones a) Follicle Stimulation Hormone (FSH)

The comparison of follicle stimulating hormone (FSH) levels across the three major groups (Control, Methotrexateinduced and Cinnamon-protected) revealed statistically significant differences, as indicated by the p-value of <0.01. In the Control group (Group A), FSH levels remained within a relatively low and narrow range (6.7 ± 0.21 to 7.75 ± 0.24), reflecting normal functioning. In contrast, the methotrexate-induced group (Group B) showed a marked elevation in FSH concentrations, with values rising progressively across subgroups (9.6 ± 0.6 to 13.9 ± 0.61). This elevation suggests impaired ovarian function or disrupted feedback regulation, which typically results in increased pituitary secretion of FSH. The consistently higher FSH levels in Group B indicate a significant biochemical disturbance associated with the methotrexate-induced condition.

The cinnamon-protection group (Group C) demonstrated intermediate FSH levels. Subgroup 2week showed slightly elevated values (8.51 ± 0.66), while 4 week and 6wee displayed lower FSH levels (6.6 ± 0.4 and 5.48 ± 0.34, respectively), approaching or even dropping below control levels. This pattern suggests a potential protective or

restorative effect of the administered intervention, helping to normalize FSH secretion. The downward trend in FSH across the cinnamon-protected subgroups implies improved ovarian hormonal regulation compared to the methotrexate-induced group.

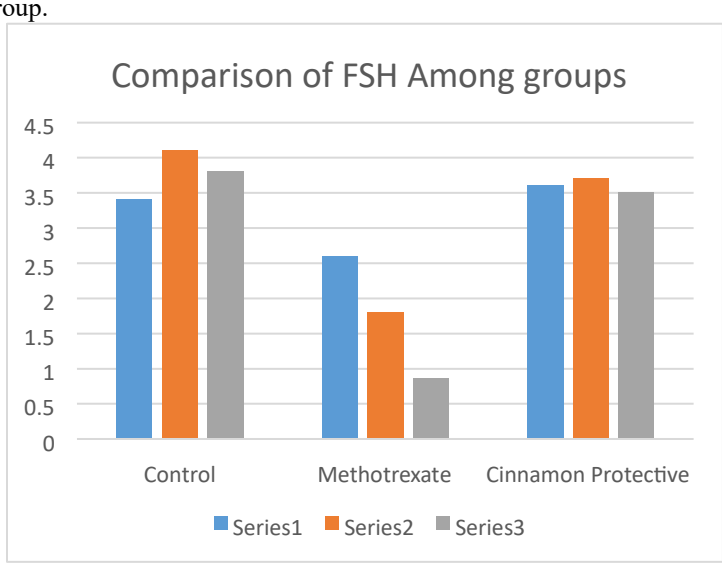

Figure 1: Serum FSH level

b) Luteinizing Hormone (LH)

Analysis of luteinizing hormone (LH) levels revealed significant differences among the study groups ($p < 0.01$). The methotrexate-induced group (B) showed markedly elevated LH levels across all subgroups, with the highest in 6 week (7.89 ± 0.53), indicating an association between the methotrexate-induced group and increased LH secretion. The control group (A) maintained moderate LH levels (4.5 ± 0.44 to 5.06 ± 0.97), representing normal baseline values. In contrast, the cinnamon-protected group (C) exhibited reduced LH levels, particularly in 4week and 6week (3.94 ± 0.52 and 3.22 ± 0.34, respectively), suggesting that the protective intervention effectively mitigated the hormonal elevation observed in the methotrexate-induced group. These findings indicate that the methotrexate-induced group significantly increases LH, while the cinnamon-protected treatment helps restore it toward normal levels.

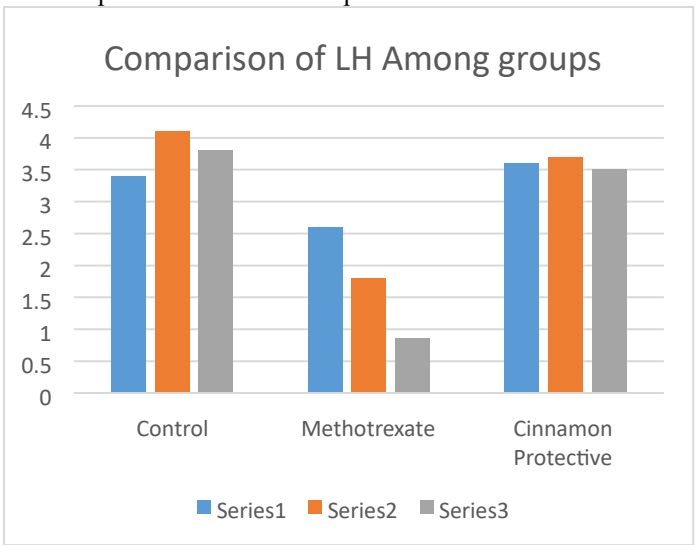

Figure 2: Serum LH level

c) Anti-Mullerian Hormone (AMH)

Analysis of AMH levels revealed significant differences among the study groups ($p < 0.01$). The methotrexate-induced group (B) exhibited substantially lower AMH levels across all subgroups, with the lowest value observed in subgroup 6 week (0.86 ± 0.13), indicating a pronounced reduction in ovarian reserve associated with the methotrexate-induced group condition. In contrast, the control group (A) maintained moderate AMH levels (3.4 ± 0.23 to 4.01 ± 0.21), reflecting normal baseline ovarian function. The cinnamon-protected group (C) demonstrated AMH levels comparable to or slightly higher than the control group (3.04 ± 0.71 to 3.67 ± 0.53), suggesting that the protective intervention effectively preserved ovarian reserve and reduced the decline observed in the methotrexate-induced group. These

results indicate that the methotrexate-induced group significantly reduces AMH, while the cinnamon-protected treatment helps maintain normal levels.

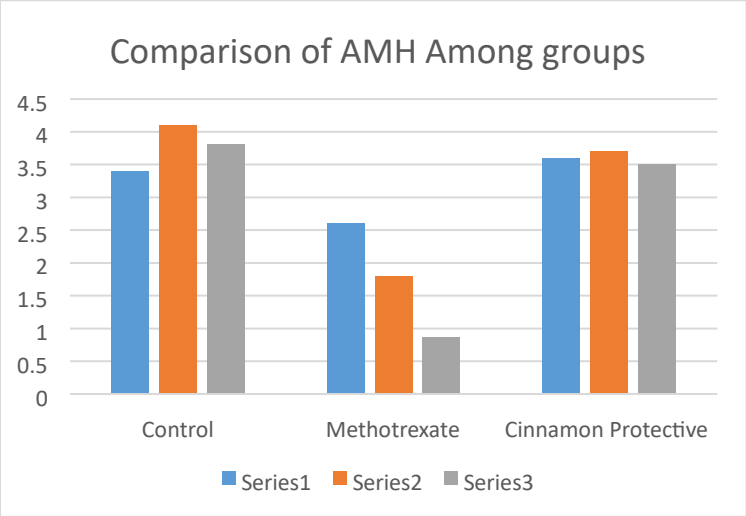

Figure 3: Serum AMH level

Table 1: Comparison of Effects on Hormones by Cinnamon Extract among Study Groups

Hormones	**Group A**		**Group B**		**Group C**		**P- value**
FSH	A1	6.7 ± 0.21	B1	9.6±0.6	C1	8.51±0.66	< 0.01
	A2	7.75 ± 0.24	B2	11.71±0.75	C2	6.6±0.4	
	A3	6.91± 0.64	B3	11.9± 0.61	C3	5.48±0.34	
LH	A1	4.82±0.18	B1	6.56±0.39	C1	5.44±0.49	< 0.01
	A2	5.06±0.97	B2	7.56±0.56	C2	3.94±0.52	
	A3	4.5±0.44	B3	9.89±0.53	C3	3.22±0.34	
AMH	A1	3.4±0.23	B1	2.64±0.35	C1	3.1±0.49	< 0.01
	A2	4.01±0.21	B2	1.89±0.12	C2	3.67±0.53	
	A3	3.84±0.58	B3	0.86±0.13	C3	3.04±0.71	

* Follicle Stimulation Hormone (FSH)
* Luteinizing Hormone (LH)
* Anti-Mullerian Hormone (AMH)

DISCUSSION

Infertility and ovarian insufficiency are just two of the problems that can arise from long-term use of chemotherapeutic drugs like methotrexate. It is becoming important to look for ways to prevent infertility in chemotherapy patients. Methotrexate is a chemotherapeutic drug and folic acid antagonist that is commonly used to treat hematological malignancies, including lymphoblastic leukemia. Because of its antimetabolite and immunosuppressive properties, it is observed in both youth and maturity[21].

Cinnamon oil, a natural antioxidant and anti-inflammatory properties[22]. The current study explores the potential effects of cinnamon oil on infertility cause by methotrexate. Because of its antioxidant qualities, cinnamon oil decreases the negative effects of the methotrexate.

In the present study, the pre- and post-experimental body weights of animals were compared across the control, methotrexate-induced, and cinnamon-protected groups to evaluate the impact of the experimental interventions. Animals in the control group showed a consistent increase in body weight from baseline to the end of the experiment, reflecting normal growth. In contrast, all subgroups within the methotrexate-induced group exhibited a marked reduction in body weight following treatment. This finding is in complete agreement with the previous study, which showed that methotrexate-induced infertility dramatically decreased body weights in comparison with control [23]. Methotrexate decreases the weight of rats as a cytotoxic agent that damages rapidly dividing cells, and its metabolic and toxic effects lead to significant toxic pathological effects on both reproduction and body weight[24]. Animals in the cinnamon-protected group demonstrated an increase in body weight as a potent antioxidant agent.

The absolute ovarian weight in the control group reflected normal conditions and healthy ovarian development. The animals in the methotrexate-induced group showed a marked and progressive reduction in ovarian weight across subgroups. This significant decline indicates that the methotrexate-induced group condition adversely affected ovarian mass, likely due to structural degeneration, follicular loss, and ovarian edema[25].

The cinnamon-protected group displayed ovarian weights that were intermediate between the control and methotrexate-induced groups. Sub-groups in 4week and 6week showed values close to those of the control group, suggesting a strong protective effect. Ovarian weight and function improved in the cinnamon-protected group due to the antioxidant activity of cinnamon oil and its role in hormone regulation. A previous study has demonstrated that the reduction in ovarian weight caused by methotrexate can be prevented by L-carnitine and folic acid because of their anti-oxidant and anti-apoptotic properties[23].

Follicle-stimulating hormone (FSH) levels were assessed. Follicle-stimulating hormone plays a critical role in reproductive health[26]. In our study, in the control group, follicle-stimulating hormone levels remained within a relatively low and narrow range, reflecting normal functioning. The methotrexate-induced group showed a marked elevation in follicle-stimulating hormone concentrations, with values rising progressively across subgroups. This elevation suggests impaired ovarian function or disrupted feedback regulation, which typically results in increased pituitary secretion of follicle-stimulating hormone. Methotrexate mainly affects gonadal steroidogenesis and causes oxidative stress, inflammation, and apoptosis in gonadal tissues. In agreement with our finding these gonadal injuries impact follicle-stimulating hormone secretion and feedback to the hypothalamic-pituitary axis[27]. Several other studies have shown that the methotrexate-induced group displays higher follicle-stimulating hormone levels[28,29].

The cinnamon-protected group demonstrated restoration of follicle-stimulating hormone levels toward normal values. Subgroups in 4 week and 6 week displayed lower follicle-stimulating hormone levels, approaching those of the control levels. This pattern suggests a potential protective or restorative effect of the administered intervention, helping to normalize FSH secretion as an antioxidant, anti-inflammatory, and antiapoptotic agent.

Luteinizing hormone (LH) is essential for initiating ovulation and sustaining reproductive cycles. The luteinizing hormone levels are key for fertility[30]. Analysis of luteinizing hormone (LH) levels revealed significant differences among the study groups ($p < 0.01$). The control group maintained moderate LH levels, representing normal baseline values. The methotrexate-induced group showed markedly elevated luteinizing hormone levels across all subgroups, indicating an association between the methotrexate treatment and increased luteinizing hormone secretion. In agreement with our finding, methotrexate causes damage to ovarian follicles which results in decrease in ovarian hormones, this leads to a loss of negative feedback on the pituitary gland, which in turn causes the pituitary to increase luteinizing hormone secretion[31].

In contrast, the cinnamon-protected group exhibited reduced luteinizing hormone levels, suggesting that the protective intervention effectively mitigated the hormonal elevation observed in the methotrexate-induced group. These findings indicate that the methotrexate-induced group exhibited significantly increased LH levels, while the cinnamonprotected as an antioxidant agent helps restore them toward normal levels. In another study, the methotrexate group had considerably higher levels of FSH and LH in contrast to the control and pinostrobin groups. On the other hand, the pinostrobin-treated group significantly improved these findings[32].

In the present study, the control group maintained normal anti-mullerian hormone levels, reflecting normal baseline ovarian function. The methotrexate-induced group exhibited substantially lower anti-mullerian hormone levels across all subgroups, with the lowest value observed in subgroup 6week, indicating a pronounced reduction in ovarian reserve associated with the methotrexate-induced condition. Consistent with the present study, references show anti-mullerian

hormone levels decreased as a result of methotrexate-induced oxidative stress, apoptosis, and granulosa cell malfunction[33,34].

The cinnamon-protected group demonstrated anti-mullerian hormone levels comparable to or slightly higher than those of the control group, suggesting that the protective intervention effectively preserved ovarian reserve and reduced the decline observed in the methotrexate-induced group. In another study it was found alpha lipoic acid increased anti-mullerian hormone levels and decreased methotrexate induced oxidative damage[35].

CONCLUSION

The present study concluded that cinnamon extract could improve the body and ovarian weight gain and biochemical alterations in rat models of methotrexate induced infertility. It is suggested that, the result could be considered promising enough in humans who are on methotrexate lead to infertility.

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