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Curative role of silymarin against reproductive toxicity of the female system during the critical periods of life

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Abstract

The current study looked into the effects of oral silymarin administration in preventing reproductive toxicity in females. 48 female rats were divided into four groups, each with 12 rats. The experimental one has four groups; GI receiving no cure for the length of the 14-day trial, GII was given 150 mg/kg body weight of silymarin via gavage, GIII receiving daily i.p. injections of co-trimoxazole at 120 mg/kg for 14 days, whereas GIV received silymarin with co-trimoxazole. Six females from each group were sacrificed at the end of the experiment, and blood was collected for hormonal and biochemical analysis. In Experimental two the remaining six females in each group, meeting with male rats. A vaginal smear was done to detect zero days of gestation by detecting sperm; females were housed separately and sacrificed after delivery. Silymarin reduced co-trimoxazole side effects, as evidenced by an increase in Final body weight, body weight gain, relative uterus weight, relative ovary weight, LH, FSH, CAT activity, and GSH concentrations, as well as lower estradiol and serum MDA concentrations, also improved fertility outcome in comparison to GIII. In conclusion, silymarin showed considerable antioxidant activity against co-trimoxazole repro-toxicity.

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Introduction

Infertility is a global issue that affects people from all walks of life. The World Health Organization defines it as the failure to conceive after 12 months or more of regular unprotected sexual contact (1). Ovulation problems, tubal injury, and sexually transmitted illnesses are some of the most common reasons for infertility in women (2). Reactive oxygen species (ROS) production can be increased due to an imbalance between antioxidants and pro-oxidants (3). This imbalance has been implicated in the pathophysiology of several female reproductive illnesses (4), including polycystic ovarian syndrome (PCOS), infertility without explanation, and endometriosis (5), as well as preeclampsia, frequent pregnancy loss, and intrauterine growth restriction (4). Oxidative stress is the most common result of toxicity;

chemical, physical, and microbiological factors all contribute to oxidative stress in the body. During oxidative stress, antioxidant and enzyme systems typically counteract the increase in reactive oxygen species (ROS) to protect the cell or tissue's integrity (6). Primary infertility is caused by disrupting the hypothalamic-pituitary-gonadal axis or a direct toxic effect on the gonads in drug-induced infertility (2). Co-trimoxazole is an antibiotic used to treat bacterial infections and is one of the most commonly used medications that affect sexual function and fertility (7). Urologists and fertility experts frequently employ co-trimoxazole, a combination of sulfamethoxazole (SMX) and trimethoprim (TMP), to treat urinary tract bacterial infections that develop before IVF treatment. It inhibits a metabolic pathway for folic acid synthesis (8). Also, it is frequently used to treat critically ill patients with infections

caused by sensitive pathogens, such as *Pneumocystis jiroveci* (9). Sulfonamides can cause significant reductions in fertility, corresponding to substantial impairments in sperm quality (10). Polyphenolic substances are possible antioxidants that can help with oxidative stress therapy and prevention (11). Silymarin, a flavonoid with strong antioxidant properties, has been isolated from the seeds of the milk thistle Silybum marianum. Since silymarin has been reported to protect against reproductive toxicity, one of its protective effects is anti-inflammation (12). It can suppress free radicals and reactive oxygen species (ROS), react with the latter, and transform them into molecules with reduced reactivity and toxicity. It also enhances the effects of physiological antioxidants like glutathione and superoxide dismutase (SOD), Previous research suggests that silymarin may protect tissues from the toxicity of routinely used anticancer drugs, and its usage during pregnancy poses no danger to the mother or the fetus (13). Furthermore, silymarin can pass through the placenta, preventing fetal weight loss caused by alcohol consumption during pregnancy (14).

The present study aimed to evaluate the potency of silymarin in ameliorating the oxidative stress of the female reproductive system during the critical period of life.

Materials and methods

Ethical approval

The study's methodology was authorized by the Scientific and Ethical Committee of the College of Pharmacy, University of Kerbala, and the approval reference number for this research is 2022An-52on February 2022.

Model for experimentation

In this experiment, 48 female rats of weight (140-160 g) were used. They were then divided into four groups, each with 12 rats, and three animals were placed in a cage. Cages were placed in the same climatic (22-26Cand 12/12 h light-cycle) and nutritional conditions for 14 days to adapt to the new environment. Silymarin powder was purchased from Sigma (S0292, USA). The groups will be treated as follows; in experimental 1 the first group (GI) rats acted as the typical control group, receiving no treatment during the study's 14-day duration. The second group (GII) received 150 mg/kg body weight of silymarin by gavage over 14 days (15). Furthermore, the third group (GIII) co-trimoxazole group, receiving daily i.p. injections of co-trimoxazole (120 mg/kg) for 14 days (16), whereas the fourth group (GIV) received (silymarin+ co-trimoxazole). Six females from each group were sacrificed at the end of the experiment (day 14), and blood was collected for hormonal and biochemical analysis, in addition to body weight changes measurement and relative organ weight measurement (g/100 g bw) (ovaries and uteri). While in experimental 2 following this period, the healthy, fertile male rat was kept in the same cage as the female rat

for the remaining six females in each group (2 male per group). After the female rat's vaginal smear, the male rat was removed from the cage, and embryonic day 0 was recorded (E0). It was then left for 21 days after the pregnancy ended; sperm-positive females were housed separately and scarified after delivery; the two-horned uteri were removed and opened by cutting longitudinally to expose the bluish implantation sites visually inspected and to identify resorption sites. Resorption sites were defined as endometrial sites with an appended amorphous masse. The number of implantation sites was defined as the result of the total number of bluish implantation sites plus the total number of resorption sites (17). Percentage of fertility determined by the following formula; the fertility%= (number of delivering females fertility/ number of mated females)/100.

Body weight changes measurement

The weights of the experiment animals were recorded at the beginning and end of the experimental period (1 and 14 days) using the following equation; The change of the body weight (g) = Final body weight (g) - Initial body weight (g).

Relative organ weight measurement

The following equation was used to calculate the relative organ weight for each of the organs studied; Relative organ weight (g/100g of body weight) = organ weight (g)/body weight (g) x 100 (18).

Hormonal assay and Biochemical profile

The concentrations of serum, FSH, LH, and estradiol were quantified using commercial ELISA kits (SUNLONG / China). According to the manufacturer's instructions, Catalase, MDA, and GSH concentrations were assessed using commercial ELISA kits (RANDOX / United Kingdom).

Fertility

The parameters were measured: Fertility percent, Number of delivering Females, Number of Total implantation sites, and Number of resorption sites.

Statistical analysis

All data was statistically examined using the Social Sciences Statistical Package (SPSS version 19). Least significant differences (LSD) were used to identify the significance of the differences between means, and $p \leq 0.05$ was considered important. One-way analysis of variance (ANOVA) was used to compare the study groups and the average of the obtained results. Obtained results were expressed as the mean plus minus standard error.

Results

Experimental one

The results in table 1 revealed a significant drop in final body weight, body weight gain, relative uterus weight, and relative ovary weight in the GIII treated group in comparison to GI group and GII group. Also increase in final body weight, body weight gain, relative uterus weight, and relative ovary weight in the GIV treated group in comparison to the GIII treated group. In this study, the serum of estradiol increased significantly, whereas LH and FSH dropped in the GIII treated group compared to the other groups (Table 2). On the other hand, there was a significant decrease in the serum of estradiol while LH and FSH increased in GIV as compared to the GIII treated group. Serum MDA concentration increased significantly compared to other groups. However, CAT activity serum and GSH concentration decreased substantially in GIII treated female rats compared to the other groups (Table 3). Also, serum MDA concentration recorded a significant decrease while CAT activity serum and GSH concentration showed an important increase in GIV as compared to GII and GIII.

Table 1: Effect of Silymarin on body weight gain and relative reproductive organ weights in co-trimoxazole treated female rats

Group	Final body weight	Body weight gain	Relative uterus weight	Relative ovary weight
GI	241.6±27.8 [b]	38.27±1.63 [b]	0.57±0.11 [b]	9.71±0.71 [b]
GII	274.1±32.4 [a]	64.29±6.42 [a]	0.73±0.16 [a]	12.13±0.12 [a]
GIII	201.3±22.8 [c]	18.36±1.44 [c]	0.42±0.02 [c]	7.32±0.29 [c]
GIV	232.4±19.5 [b]	33.49±2.32 [b]	0.55±0.13 [b]	8.96±0.15 [b]
LSD	28.18	14.38	0.12	1.41

Table 2: Effect of silymarin on serum reproductive profile in Co-trimoxazole treated female rats

Group	Estradiol (pg/ml)	LH (µIU/ml)	FSH (µIU/ml)
GI	64.27±3.61 [b]	3.41±0.13 [b]	4.41±0.36 [b]
GII	48.14±4.02 [c]	4.87±0.62 [a]	6.31±0.79 [a]
GIII	81.55±6.13 [a]	1.45±0.09 [d]	2.61±0.08 [c]
GIV	57.83±4.63 [bc]	2.85±0.21 [c]	4.21±0.18 [b]
LSD	16.08	1.37	1.53

Experimental two

All female rats exposed to GIII after mating with males failed to be pregnant and had lower pregnancy rates, fewer implantation sites, fewer births, and a higher total number of resorptions out of an unlimited number of implantations than other groups. Still, these parameters improved in combination with GIV as compared to GII and GIII (Table 4).

Table 3: Effect of silymarin on serum oxidant-antioxidant status in Co-trimoxazole treated male rats

Group	MDA (mmol/ L)	CAT (pg/uL)	GSH (ng/L)
GI	19.37±3.11 [b]	53.61±0.43 [b]	49.36±0.26 [b]
GII	17.12±2.23 [c]	64.31±0.37 [a]	61.187±0.65 [a]
GIII	22.15±3.17 [a]	43.49±0.59 [c]	38.11±0.18 [c]
GIV	19.41±3.64 [b]	52.12±0.61 [b]	50.91±0.68 [b]
LSD	2.18	8.54	10.13

Table 4: Effect of silymarin on serum oxidant-antioxidant status in co-trimoxazole treated male rats

Group	No. female	Delivering female	Fertility %	Mean number ± SE		
				Implantation sites	Birth	Resorption Site
GI	6	5	83.33	11.3±1.07 [b]	9. 3±1.17 [b]	2.0±0.02 [a]
GII	6	6	100	14.6±1.02 [a]	13.6±0.11 [a]	1.0±0.01 [b]
GIII	6	3	50	4.5±0.23 [c]	2.5±0.13 [c]	2.0±0.01 [a]
GIV	6	5	83.33	10.2±1.53 [b]	9.2±1.18 [b]	1.0±0.01 [b]
LSD				3.19	4.15	0.87

Discussion

Because co-trimoxazole causes cellular antioxidant defense capacity overloading, decreased body weight gains in co-trimoxazole-treated female rats could be linked to increased oxidative stress (19). Reactive oxygen species (ROS) begin to destroy cellular macromolecules such as DNA, lipids, proteins, and cellular amino acid reserves at this period. As a result of the change in oxidative status, the amino acid pool will be extensively oxidized, while lipids and proteins will be significantly impacted in conjunction with cytotoxicity (20). Decreased ovarian and uterine weights in co-trimoxazole-treated female rats were investigated in this study because Co-trimoxazole is an

exogenous stressful factor that may act as an inhibitory effector on the hypothalamus-pituitary-ovarian axis, whereas silymarin has been investigated alone or in combination with co-trimoxazole as an exogenous ameliorating factor that may act as an inhibitory effector on hypothalamus (21).

In comparison to the GIII treated group, silymarin reduced the harmful effect of co-trimoxazole, as evidenced by an increase in Final body weight, body weight gain, relative uterus weight, and relative ovary weight in the GIV treated group. This could be due to silymarin's ability to stabilize the membranes of hepatocytes, resulting in hepatocyte protection and better liver function (22). Furthermore, the stimulatory effect of silymarin on RNA and protein synthesis could lead to better growth and body weight gain (23). It could also be linked to silymarin's stimulatory effect on protein synthesis. Researchers hypothesized that silymarin acts as an RNA polymerase-I stimulant, allowing it to control rRNA transcription. The biosynthetic rate of structural and functional proteins could be improved by increased ribosome production and quicker protein and DNA biosynthesis. Furthermore, silymarin's stimulatory impact may result in more transporters and enzymes, which could enhance the activities of various bodily cells (24). The improvement in the pituitary-gonadal axis is attributed to the improvement in ovarian and uterine weights in this study's silymarin-treated group (25) and stated that the herbal extracts' gonadotrophic-like activities can affect various biological parameters, such as increased ovarian and uterine relative weight, ovulation induction, steroidogenesis, and protein biosynthesis. According to reports, natural herbal antioxidant sources have been used to treat abnormal hormone functioning caused by oxidative stress (26). In this study, the serum of Estradiol increased significantly, whereas LH and FSH dropped in the GIII group compared to the other groups.

Toxic chemicals may cause cell membrane lipid peroxidation, cellular protein oxidation, or DNA damage (27), with any disturbance of these processes at any functional level potentially leading to infertility (28). As a result, any impairment of gonadotrophin secretion from the anterior pituitary could compromise follicular cell proliferation, oocyte survival, and steroid synthesis (particularly estradiol). The decreased levels of LH and FSH in the GIII group overproduction of free radicals is one of the mechanisms by which toxicants can cause side effects, with excessive accumulation of free radicals leading to oxidative damage in reproductive tissue (29) and thus impairment of reproduction (30), with pathological consequences due to lipid damage, protein synthesis inhibition, or ATP depletion (31). In this study, the decrease in relative weights of reproductive organs (ovaries and uteri) in the co-trimoxazole group female rats indicate co-trimoxazole nephrotoxicity. Because the importance of the ovaries is highly dependent on the mass of folliculogenesis (32), the significant reduction in reproductive organ weights caused by co-trimoxazole can be attributed to a decreased number of follicles, accompanied by a decrease in FSH secretion. Furthermore, this study's high amounts of estradiol could result from elevated gonadotropin. During follicular growth and maturation, it is predominantly induced by follicular development (33).

On the other hand, there was a significant decrease in the serum of Estradiol while LH and FSH increased in GIV as compared to the GIII treated group. According to several researchers, improving lipid peroxidation and oxidative stress, following negative changes in the oxidant-antioxidant balance, is the most plausible route to mitigate the unfavorable effects of co-trimoxazole (34). Restoring oxidant/antioxidant balance is an intriguing technique to minimize the adverse impact that toxicants might cause. The protective benefits of numerous antioxidants and naturally occurring compounds against reproductive toxicity may be attributable to their ability to scavenge free radicals (35). The treatment of silymarin significantly reduced the production of reproductive hormones, emphasizing its involvement in reducing oxidative damage caused by co-trimoxazole. On the other side, silymarin may help to normalize ovarian function, allowing for a greater preference for reproductive activity. It was confirmed that oxidative stress reduction and lipid constituent regulation were major factors controlled by natural products, boosting female fertility (33). Endogenous and exogenous influences can alter the manufacture and activity of these hormones (36).

In comparison to other groups, serum MDA concentration increased significantly, although CAT activity serum and GSH concentration decreased substantially in GIII co-trimoxazole treated female rats. Significant increases in serum MDA and decreases in serum antioxidant concentrations could be related to pathological alterations in liver tissue, as the liver is the primary organ for antioxidant synthesis and oxidant metabolism. The oxidant-antioxidant imbalance could be confirmed by pathological alterations in the liver (37). Because of its ability to interact with lipoproteins, a raised serum MDA level is also regarded as an essential biomarker or measure for the degree of oxidative stress (38), where there was a positive connection between serum MDA levels and lipid peroxidation (39). Membrane lipid peroxidation and enhanced reactive oxygen species production may cause oxidative damage in the co-trimoxazole-treated group, which may surpass the capacity of the cell's exogenous antioxidants. To combat oxidative stress and prevent lipid peroxidation, glutathione works in concert with other antioxidants such as vitamin e (40). The decreased glutathione levels in the co-trimoxazole administrated group could be related to the severe use of antioxidants in scavenging the free radicals generated during the metabolism of co-trimoxazole (41).

Also, serum MDA concentration recorded a significant decrease while CAT activity serum and GSH concentration showed an important increase in GIV as compared to GIII. Because silymarin has induced action for liver tissue to

overexpress endogenous antioxidant synthesis, the results demonstrated that silymarin has an ameliorating role. The current data show that silymarin has significant pharmacological value as an antioxidant and an inducer of endogenous antioxidants in intact animals and co-trimoxazole-induced toxic female rats. They discovered that silymarin was an exogenous antioxidant and induced endogenous antioxidant synthesis. Silymarin-treated rats had higher free radical scavenging activity due to enhanced catalase activities and glutathione peroxidase levels (42). Catalase and glutathione peroxidase have been mentioned as enzymic antioxidant defense mechanisms against reactive oxygen species (43) and the repair of membrane lipid peroxidation following silymarin treatment (44). As a result, silymarin protects cellular membrane lipids from further damage. This was in line with our findings. Silymarin treatment could efficiently reverse the depletion of non-enzymic antioxidants induced by co-trimoxazole because silymarin has been shown to maintain GSH homeostasis (45), and this could explain the enhanced glutathione levels seen in silymarin-treated groups. Elevated serum MDA levels might be associated with hyperlipidemia and improve lipid peroxidation (46). Therefore, the hypolipidemic effect of silymarin could be attributed to the decline of MDA levels. Reducing MDA levels in female rats treated with silymarin could be a favorable sign of hypolipidemia (39). The difference in serum MDA concentrations between female rats treated with silymarin and those treated with co-trimoxazole demonstrates silymarin activity. Silymarin's protection appears to be linked to a counteracting process in the fight against free radicals (47). Furthermore, silymarin's scavenging action may be due to its activity against lipid peroxidation, where significant amounts of metabolites and free superoxide radicals are produced in mitochondria during normal metabolism and oxidative stress. Still, the available or active antioxidant action is the crucial difference between the two states (46).

Many studies have shown that silymarin has significant efficacy by increasing GSH synthesis and Gss gene expression in hepatocytes (48). In most body cells, particularly hepatocytes, the Gss gene encodes mitochondrial and cytoplasmic glutathione synthetase, essential for glutathione synthesis (46). Furthermore, raising glutathione levels may activate and increase the activity of glutathione peroxides, which have antioxidant and free radical scavenging properties (49).

In terms of female fertility, all female rats exposed to GIII after mating with males failed to be pregnant and had lower pregnancy rates, fewer implantation sites, fewer births, and a higher total number of resorptions out of an unlimited number of implantations than other groups. Still, these parameters improved in combination with GIV compared to GIII.

Primary infertility is caused by disrupting the hypothalamic-pituitary-gonadal axis or a direct toxic effect on the gonads in drug-induced infertility. As a result, any factor that affects the hypothalamus-pituitary-gonadal axis may cause female infertility, which may be caused by failures at various stages of reproduction, such as ovulation, fertilization, and embryo development (50). On the other hand, exposure to various environmental toxins may result in decreased fertility, reduced follicular growth, and even ovarian tissue damage (51). One of the most common medicines that impact sexual function and fertility potential is co-trimoxazole (52). In previous research, it has been shown to produce considerable reductions in fertility (10) and a decrease in pregnancy rate and litter size (53). Co-trimoxazole is a mixture of sulphamethoxazole and trimethoprim (5 parts to 1 part), inhibiting folic acid synthesis and metabolism. Sulfur medicines, such as sulphasalazine, have been used to treat ulcerative colitis and Crohn's disease for many years, and they are known to affect fertility. Co-trimoxazole causes oligospermia, sperm motility problems, and morphological alterations in the sperm, as well as a low pregnancy rate (54,55).

The ability of silymarin to prevent the action of free superoxide radicals produced by co-trimoxazole treatment could be attributed to the beneficial effects of silymarin on fertility outcomes described in this study. Because free radicals are produced due to increased lipid peroxidation in cell membranes and increased hepatic cell metabolism, they activate ribosomal RNA biosynthesis and stimulate protein biosynthesis (56). Furthermore, whereas silymarin treatment raises GSH and FSH levels, this increase may be counterbalanced by free radicals in the developing follicle (32). Silymarin is also well tolerated by pregnant women at large doses, with no toxicity documented for both mother and fetus (57). Despite its capacity to pass through the placenta (15), silymarin has no toxicity for the fetus (15) and can reduce fetal weight loss caused by any ingestion during pregnancy (15). As a result, antioxidants such as silymarin can aid in improving fertility rates (57).

Conclusion

Our findings demonstrated that silymarin has significant antioxidant activity, allowing it to scavenge the extra free radicals caused by co-trimoxazole repro-toxicity, inactivate them, and heal the damage.

Acknowledgments

Conflict of interests

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الدور العلاجي للسيليمارين ضد السمية الإنجابية للجهاز الأنثوي خلال الفترات الحرجة من الحياة

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الخلاصة

تهدف هذه الدراسة إلى التحقق من تأثيرات التجريع الفموي للسيليمارين للوقاية من سمية الجهاز التكاثري في الإناث. تم تقسيم ٤٨ أنثى من الجرذان إلى أربع مجموعات لكل منها ١٢ جرذ، وتم إعطاء العلاجات التالية: في التجربة الأولى اعتبرت المجموعة الأولى كمجموعة سيطرة لم تستلم أي علاج طيلة فترة التجربة (١٢ يوم)، أما المجموعة الثانية مجموعة السليمارين فلقد جرعت فمويا بالسيليمارين، مجموعة الكوتريموكسازول حصلت حقن الكوتريموكسازول عن طريق الوريد مرة واحدة يوميًا لمدة ١٤ يوما، بينما حصلت المجموعة الرابعة على سيليمارين والكوتريموكسازول. بعد نهاية التجربة تم التضحية بستة إناث من كل مجموعة وتم الحصول على الدم للتحاليل الهرمونية والكيميائية الحيوية. أما التجربة الثانية فلقد تضمنت ستة إناث من كل مجموعة وضعت مع ذكور الجرذان في نفس المكان. تم استخدام المسحات المهبلية لتحديد حالات الحمل في اليوم صفر من الحمل، تم عزل الإناث إيجابية المسحة بوجود الحيوانات المنوية وتم التضحية بهن بعد الولادة. أظهرت نتائج الدراسة الحالية أن السيليمارين عمل على تقليل التأثيرات الجانبية للكوتريموكسازول كما يتضح من زيادة وزن الجسم النهائي، وزيادة وزن الجسم ووزن الرحم النسبي، ووزن المبيض النسبي، والهرمون المنشط للجسم الأصفر، والهرمون المحفز للقند، ونشاط إنزيم الكتاليز وتراكيز الكلوتاثيون، بالإضافة إلى انخفاض تراكيز الاستراديول ومستوى المالوندالديهايد، بالإضافة الى تحسن نتائج الخصوبة مقارنة مع المجموعة الثالثة. نستنتج من الدراسة الحالية أن للسيليمارين نشاطا مضادا للأكسدة ضد سمية الكوتريموكسازول.