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Original article

The Possible Protective Role of Selenium Supplementation Against Some Hormone Alterations Induced by Diclofenac Sodium in Female Rats

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Abstract
Diclofenac sodium (DFS) has affected the tests for serum hormones. Crucially, prior studies indicate that diclofenac functions as a thyroid protein antagonist. An important minor mineral that guards and prevents oxidative harm is selenium (Se). Aims: The goal of this study is to explore the negative impact of DFS, Se, and their combination on: some levels of serum hormones such as" thyroid stimulating hormone (TSH)", "triiodothyronine (T3)"," thyroxin hormone (T4"(, "follicle-stimulating hormone (FSH)", "luteinizing hormone (LH)" in female albino rats. Methods: Twenty albino female rats 170-200 grams, were randomly distributed into 4 groups of 5 rats. Untreated (1 ml distilled water for 21 days), Se cohort (0.25 mg per kg for three weeks), DFS cohort (10 mg per kg for 2 weeks), and protective cohort (Se + DFS). The protective group received Se for seven days, followed by Se and DFS two hours apart over two weeks. Results: The mean values revealed a notable decline in levels of T3, FSH, and LH, as well as a rise in the amount of TSH, PRL, and progesterone in the DFS group. Meanwhile, rats in the Se group revealed no notable variations in levels of TSH, T3, and FSH, but an increase in levels of T4, PRL, progesterone, and a decrease in LH and estrogen. The administration of Se with DFS was able to reduce and alleviate the harmful effects of DFS on most of the measured parameters. In conclusion, the findings of this research showed that Se effectively reduced thyroid hormone disorders and changes in female sex hormones in female albino rats.
Keywords. Diclofenac Sodium, Selenium, Female Sex Hormones, Thyroid Hormone.

Introduction

The most commonly recommended medicines are Nonsteroidal anti-inflammatory medications (NSAIDs) have anti-inflammatory, antibacterial, and alleviating effects. In wealthy nations, almost thirty percent of people regularly use them [1]. Diclofenac Sodium (Voltaren) (DFS) is among the most extensively prescribed NSAIDs in the world. It is used mainly to relieve symptoms across multiple clinical indications, including inflammation, pain, osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis [2]. DFS has effects on. testing for thyroid function. Crucially, earlier studies indicate that DFS functions as a thyroid protein antagonist [3].

It has been demonstrated that several NSAIDs belonging to the phenyl acetic acid group lower blood thyroxine concentrations and the circulating thyroxine indices in euthyroid individuals [4]. Previous results also showed that DSF inhibited the binding of T3 and T4 competitively to the binding protein [5]. Whole-body concentrations of T4 were considerably lowered after DFS administration. in zebrafish embryos/larvae [6]. THRs are highly expressed in both males and females, suggesting their importance in reproductive physiology. In females, THRs regulate the growth of uterine cells, ovaries, endometrial granulosa, placental tissue, etc. [7]. According to Koyyada and Orsu [8], both of these conditions can cause erratic menstruation and ovulatory phases, which might impact fertility. A variety of reproductive defects, including polycystic ovarian syndrome, irregular menstruation, fertility problems, and compromised egg production, fertilization, or placement that results in premature births as well as problems with pregnancy, may be caused by disruption in thyroid control of the hypothalamic pituitary-gonadal center [8, 9].

The vital trace element selenium (Se) prevents oxidative damage [10]. Selenoproteins contain selenocysteine, which is an antioxidant [10,11]. Glutathione peroxidases, which lower the levels of thioredoxin reeducate, hydrogen peroxide, and phospholipid hydroperoxides, which preserve inside of cells redox condition and aid in the regeneration of antioxidant enzymes; and selenium-containing proteins may shield endothelial cells from selenium-deficient people [10].

Se exerts an important part in the system that protects against antioxidants as it is a constituent of the enzyme glutathione which plays a crucial part in the glutathione antioxidant pathways that detoxify lipid peroxides and protect cellular and subcellular membranes from damage caused by oxygen radicals Furthermore, selenium has demonstrated to have a positive effect on various bodily systems, including the immune system, thyroid, brain, heart, and reproductive systems [11]. This research aims to assess the potential protective impact of selenium supplementation against various hormone changes induced by DFS in female rats.

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Methods

Chemicals

DFS 50 mg was gotten hold of from the Algerian company Hikma, while Se 200 µg was obtained from the American company Carlson. Each ingredient was powdered separately and diluted in 10 milliliters of distilled water.

Animals

Animal experiments were approved by the Libyan Authority for Scientific Research - General Commission for Bioethics and Biosafety - Biomedical Ethics Committee of Al-Bayda Campus, University of Omar Al-Mukhtar – Libya. (NBC: 007. A. 24. 5)The investigation included 20 adult albino mice (they weighed between 170 and 200 g). Provision of the animals was facilitated by the Animal Center, Department of Zoology, Science Faculty, Omar Al-Mukhtar University, and housed in metal cages at an ambient temperature of 21 ± 3 °C. For three weeks, the animals were acclimatized to laboratory settings while being fed conventional feed and water.

Experimental Procedure

A total of four groups were formed, with five rats randomly allocated to each group. The first group, a control group, fed with a standard feed and distilled water, was administered to the animals. The second group, Se group fed a daily oral dose of Se (0.25 mg per kg) was administered each morning for 3 weeks [12]. The third group, DFS group received DFS via oral gavage at 10 mg per kg every day for 14 consecutive days [13]. The last group, Se & DFS group, for the first 7 days, rats were treated with Se at 0.25 mg per kg using a gavage tube. In the following two weeks, the animals continued to receive selenium at the same dose in the early morning, after a two-hour interval, DFS was administered orally at 10 mg per kg body weight.

Biochemical analysis

After the experiment, the rat was put under anesthesia with mild ether, and blood was immediately euthanized. Blood samples were collected into test tubes and kept for three hours to complete clotting. The clotted blood samples were centrifuged at 3,000 rpm for 10 minutes, and clear serum samples were extracted for biochemical analysis.

The CL-series TSH test is a Chemiluminescent immunoassay (CLIA) for the quantifiable detection of TSH in an individual's serum. The basis of the approach was disclosed by [14]. The amount of T3 and T4 in the serum was measured via an industrial kit provided by Coat-A-Count, a company based in Los Angeles, US [15]. A commercial reagent provided by Coat-A-Count FSH IRMA, a laboratory assay kit sourced from Los Angeles, USA, was employed to quantify serum FSH and LH levels [16]. The analyses of the serum prolactin concentration were performed utilizing Ichroma (Boditech Med Inc.) [17]. Progesterone was estimated according to [18]. Serum Estradiol hormone (E2) Serum estimates a leptin enzyme immune assay or ELISA kit, was used to test RBP4 in blood quantitatively [19].

Methods of statistical assessment

Results were reported as the mean ± standard error of the mean (SEM), based on five replicates per group. To assess statistical significance among the different experimental groups, one-way analysis of variance (ANOVA) was conducted using Minitab software (version 17). After which, the Tukey method is used to provide more precise p-values for group comparisons. P-values < 0.01 showed significant differences.

Results

Table (1) documents the following results: No discernible changes in the Se group's mean TSH value (0.01 ± 0.001) in comparison to the control (0.01 ± 0.001). Conversely, the DFS group's mean TSH readings (0.02 ± 0.003) were much greater than those of the normal animals. In the meantime, there were no discernible effects in TSH activity between the Se + DFS group (0.01 ± 0.001) and control animals. Otherwise, the presence of Se with DFS reduced the increase in TSH levels versus the DFS group. In comparison to the untreated animals (0.72 ± 0.04), the Se group (0.77 ± 0.04) showed a non-significant impact in T3. whereas, the DFS group's mean T3 measurements rose significantly (1.63± 0.07) in contrast to the control. However, concerning the control, the Se + DFS treatment caused a highly significant decline (0.42 ± 0.05). Furthermore, it dropped as opposed to the DFS animals. The T4 level data were displayed in the same Table. The Se group's mean T4 value (2.47 ± 0.03) was slightly higher than the control group's (2.12 ± 0.06), according to the results. Conversely, the DFS group had a substantial drop (P < 0.01) relative to the untreated animals (1.63 ±0.07). Simultaneously, there were no appreciable variations between the control (2.12 ± 0.06) and the Se + DFS (2.06 ± 0.12).

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Table 1. It shows average values of thyroid-related hormones (TSH, T3, and T4)in the serum of female rats given Se, DFS, and their combined treatment compared to the control group.

Parameters	Experimental groups			
	Control Mean ± SEM	**Se Mean ± SEM**	**DFS Mean ± SEM**	**Se + DFS Mean ± SEM**
TSH (µl U/ml)	0.01 ± 0.001^{B}	0.01 ± 0.001^{B}	0.02 ± 0.003^{A}	0.01 ± 0.001^{B}
T3 (ng/ml)	0.72 ± 0.04^{A}	0.77 ± 0.04^{A}	1.63 ± 0.07^{C}	0.42 ± 0.05^{B}
T4 (µg/d L)	2.12 ± 0.06^{B}	2.47 ± 0.03^{A}	1.63 ± 0.07^{C}	2.06 ± 0.12^{B}

The data are presented as means ± SEM, with a sample size of 5 for each group. Significant differences (P < 0.01) between averages in the same row are indicated by different elevated script letters (A, B & C). While averages with identical elevated script letters indicate no significant change

Table (2), which shows the data for serum FSH levels, showed no significant changes (P < 0.01) in the Se group (2.29 ± 0.24) in contrast to the group serving as the control, 2.26 ± 0.20. However, the DFS group (0.90 ± 0.07) and Se + DFS group (1.15± 0.09) showed an extremely significant reduction (P < 0.01) relative to the normal animals. The findings of the LH level are documented in Table 2. Comparing the Se group (0.68 ± 0.17) to the group used for control (1.08 ± 0.01), the results exhibited a substantial reduction (P < 0.01). Additionally, the mean value of LH (0.07 ± 0.01) decreased significantly (P < 0.01) after DFS therapy against to the control. However, statistically, the untreated animals and the Se +DFS group (1.02 ± 0.08) did not differ significantly. In the same Table, display the average PRL level values for both the control and the experimental groups. When contrasted with the normal group (2.75 ± 0.12), there was no notable rise in the Se group. (3.25 ± 0.17), and in the Se+ DFS group (3.59 ± 0.12). On the other hand, relative to normal animals, the DFS group had a considerable rise (3.98 ± 0.34). Additionally, there were no discernible variations in the mean progesterone levels among the treated and untreated animals. Recorded in the normal group (15.14 ±0.19), (15.32 ± 0.20) in the Se group, (15.92 ± 0.21) in the DFS-treated animals, and (15.92 ± 0.21) in the Se + DFS animals. The data in Table 2 show the results of estradiol hormone, a non-significant drop in the average value of the Se group (148.32 ± 1.45) and Se + DFS group (151.96 ± 1.87) in comparison to the normal animals (157.14 ± 3.60). Conversely, animals reserved for the DFS group showed a non-significant rise (163.92 ± 2.04) relative to the normal group.

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Table 2. ***This table shows the mean values of serum FSH, LH, and PRL of female rats given Se, DFS, and their combined treatment in comparison to the control***

Parameters	Experimental groups			
	Control Mean ± SEM	**Se Mean ± SEM**	**DFS Mean ± SEM**	**Se + DFS Mean ± SEM**
FSH (m IU/ml)	2.29 ± 0.24^{A}	2.26 ± 0.20^{A}	0.90 ± 0.07^{B}	1.15 ± 0.09^{B}
LH (m l U/ml)	1.08 ± 0.01^{A}	0.68 ± 0.17^{B}	0.07 ± 0.01^{C}	1.02 ± 0.08^{AB}
Prolactin (ng/ml)	2.75 ± 0.12^{B}	3.25 ± 0.17^{AB}	3.98 ± 0.34^{A}	3.59 ± 0.12^{AB}
Progesterone (ng/ml)	15.14 ± 0.19^{A}	15.32 ± 0.20^{A}	15.92 ± 0.21^{A}	15.92 ± 0.21^{A}
Estrogen (Oestradiol) (pg /ml)	157.14 ± 3.60^{AB}	148.32 ± 1.45^{B}	163.92 ± 2.04^{A}	151.96 ± 1.87^{B}

The data are presented as means ± SEM, with a sample size of 5 for each group. Significant differences (P < 0.01) between averages in the same row are indicated by different elevated script letters (A, B & C). While averages with identical elevated script letters indicate no significant change. The data are presented as means ± SEM, with a sample size of 5 for each group. Significant differences (P < 0.01) between averages in the same row are indicated by different elevated script letters (A & B). While averages with identical elevated script letters indicate no significant change

Discussion

The current study involves NSAIDs, which are often used in fields of pain treatment, including surgical operations, and alleviation from dysmenorrhea and heavy monthly bleeding, for their dual actions as painkillers and reduction of bleeding during periods of loss [20]. They have been widely used to manage many chronic diseases, including various joint disorders, like axial spondyloarthritis, autoimmune arthritis, and chronic joint wear [21]. Misuse of NSAIDs can impede normal reproductive processes, which can lead to temporary infertility, which is reversible [22]. Nonetheless, these drugs may lead to various side effects, which can vary from moderate to severe. There is less scientific evidence on the impact of NSAIDs on hormone levels in women. DFS is an example of these drugs. Selenium is an important trace mineral that protects against oxidative damage [8].

As a result, this study explored the detrimental effects of DFS, Se, and their combination on various blood hormone levels in female albino rats, including T3, T4, TSH, FSH, and LH. In the current study, the DFS group exhibited significantly higher mean TSH and T3 levels in comparison with the control group. T4 levels decreased significantly. DFS has been shown to impact thyroid function testing. Importantly, earlier data indicate that DFS is a thyroid receptor antagonist [3]. Certain NSAIDs of the phenylacetic acid group have been demonstrated to reduce serum T4 levels and the free T4 index in euthyroid subjects [4]. Previous

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research has shown that DSF hinders the competitive binding of T3 and T4 to the binding protein [5]. Treatment with DFS dramatically lowered entire-body circulating T4 levels in embryonic and larval zebrafish [6]. However, these observations disagreed with a study by [23], who found that non-linear white rats received DFS intragastrically (10 mg/kg and 12 mg/kg). There are no statistically important changes. in influence on thyroid hormone levels was found. DFS (12 mg/kg) did not generate significant changes in T3, T4, or TSH, and these variations may pertain to the time of trial, done over 42 days, and under the settings of experimental osteoarthritis on the background of hypothyroidism.

In the current study, the presence of selenium with DFS reduced the increase in TSH levels relative to the DFS-treated animals and led to a substantial lowering in T3, significantly dropped compared to the DFS group. Meanwhile, there were no noteworthy changes in T4 level between Se + DFS and compared to the control group. Se catalyzes every recognized selenoenzyme, including “iodothyronine deiodinases”, which are required for the stimulation and suppression of the thyroid gland's hormones T4 and T3., accordingly [24]. The effect of Se was more pronounced in female mice because subsequent Se injection after iodine exposure resulted in fewer changes in thyroid structure, maintaining the typical features of the gland's function [25].

Previous studies state that Se plays a critical function in thyroid hormone synthesis. It has a crucial role in thyroid hormone production. Se-deficient diet reduces T3 levels while increasing T4 levels and decreasing the T3/T4 ratio in plasma. These implications may have an impact on the rate of growth because T3 is the bioactive version of T4. version of T4, which is noted for participating in growing activities. T4 is activated by the enzyme “5-iodothyronine deiodinase”; among the final proteins is this selenoprotein to be damaged by Se deprivation; the results of Se and DFS co-administration support this hypothesis about a possible defensive role (it is required for selenoprotein synthesis) [26].

In the present investigation, DFS medication resulted in lower FSH and LH levels, but higher PRL levels than the normal animals. There were no important variations in progesterone values between the treatment and control groups, and estrogen (estradiol) values in animals assigned to the disease-free survival group showed a non-significant increase in contrast to the control [27]. The expression of FSH r and LH r genes increased significantly after NSAID exposure. In contrast, several studies found that therapy using NSAIDs produces a drop in the two blood FSH and LH concentrations [28] and with DFS at doses (of 1 mg per kg) and (5 mg per kg) for 5 weeks in the female rats, or non-substantial variations in LH and FSH values [29].

Serum “progesterone and estrogen” levels were measured in this study to thoroughly summarize the impact of DFS regarding the genital tract of females’s total blood hormones. Estrogen is a major hormone in the female reproductive system that regulates a variety of physiological functions. It stimulates the development of the ovarian follicle, increases the mid-cycle surge of gonad hormones, changes the consistency of the cervical fluid to aid sperm transport, and preparations the uterine endometrium lining for possible implantation of an embryo [30].

A previous study found a significant positive connection between TSH and PRL. Saxena *et al.* also investigated the association between TSH and PRL in infertility and found a strong positive connection [31]. Previously, a higher frequency of “hyperprolactinemia” in infertile women and discovered a 4:1 positive association between hypothyroidism and hyperprolactinemia [32]. Other investigations of patients with subclinical hypothyroidism and primary hypothyroidism discovered a good connection between TSH and PRL [33]. Also, DFS did not influence plasma gonadotropin levels; nevertheless, its administration was associated with a substantial drop in gonadotropin-releasing hormone and testosterone, as well as a large rise in prolactin secretion [34]. There are references in the literature regarding the central [35], and peripheral [36] effects of DFS. Chronic DFS administration causes oxidative stress, and thus the generation of reactive oxygen species. which can surely pass the blood-brain barrier and disrupt gonadotropin-releasing hormone output in the hypothalamus. In women's infertility, inflammation is thought to be one of the repercussions of an imbalanced antioxidant enzyme system. Oxidative stress stimulates all pathways that activate the synthesis of the inflammatory mediators [37].

As a result, there can be a favorable correlation between oxidative stress and inflammation [38]. A recent cross-sectional study in Basrah City was conducted among participant women taking DFS and found that the level of LH substantially rose and had an important impact on the prolactin level during the menstrual cycle [39]. DFS is widely known for its anti-inflammatory actions. Chronic diseases are often treated with medication over an extended period. An extended dose of DFS has been linked to oxidative stress, which has a pathogenic relationship with inflammation [37].

The anterior pituitary gland produces thyroid hormones, prolactin, FSH, and LH, which have a substantial impact on female fertility. Hormonal testing is a crucial part of the infertility diagnosis process. Ovulation is blocked by gonadotropin-releasing hormone, which contributes to infertility when there is high prolactin. Furthermore, as gonadotropin-releasing hormone output decreases, so does LH and FSH secretion. As a result, gonadal steroidogenesis is blocked, and gametogenesis does not rise [40].

Prolactin suppresses two hormones necessary for ovulation: FSH and GnRH. When prolactin levels are high in the blood (hyperprolactinemia), a female will not ovulate, and this leads to infertility. Prolactin inhibits two hormones required for ovulation: FSH and GnRH. High prolactin levels in the blood (hyperprolactinemia) prevent a female from ovulating, resulting in infertility [41]. Prolactin suppresses two hormones necessary

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for ovulation: FSH and GnRH. High prolactin levels in the blood (hyperprolactinemia) prevent a female from ovulating, resulting in infertility.

According to a study found that amenorrhea occurs in hypothyroidism due to hyperprolactinemia caused by a deficiency in estrogen's positive feedback on LH, as well as a reduction of both LH and FSH levels [42]. It also discovered an important decline in circulating LH during the follicular, ovarian, along luteal stages in Hyperprolactinemic women with both initial and subsequent infertility [42]. Women suffering initial infertility reported considerably lower circulating FSH concentration during the ovulation period. Similarly, hyperprolactinemic women exhibiting subsequent infertility experienced a considerable decrease in blood FSH levels throughout the luteal phase .Selenium treatment raises FSH, LH, progesterone, and prolactin levels above those of DFS patients, bringing them closer to those of controls. This is consistent with research showing that Se reduces serum lead levels and regulates reproductive hormones [43].

Se may have increased endogenous glutathione activity, which could explain the increased concentration of these hormones. Selenium increases the amount of glutathione, an endogenous antioxidant that helps prevent fat oxidation and the resulting cell damage [44]. Meanwhile, the present study found no statistically significant variations in mean TSH and T3 levels between the Se and control groups. The results indicated a slight rise in the average T4 levels. Selenium supplementation dramatically altered immunological indicators, improving lambs' defensive mechanisms. Conclusions of this research suggest that long-lasting selenium supplementation benefits sheep by raising their immunity, and thus enhancing their performance [45].

Authors who tested short-term selenium treatments in sheep found similar outcomes [46,47], significantly higher T3 levels in the selenium-supplemented group were consistent with [48]. They discovered that lambs given selenium supplementation produced more thyroid hormone. Selenium has a vital function in thyroid hormone metabolism. 5-iodothyronine deiodinase is a selenium-dependent enzyme [49]. Thus, selenium supplementation can raise T3 concentrations by improving T4 deiodination towards its functional component T3; a considerable rise in prolactin levels observed in Se-treated animals is possibly linked to the effect of selenium on enhancing prolactin production and secretion. [50]. The hormonal analysis demonstrated a significant progressive improvement in progesterone concentration in the Se + DFS group relative to the DFS-treated rats, which is according to previous findings [51,52].

The results obtained are most likely related to the effect of Se on improving ovarian reactivation by stimulating progesterone synthesis by the corpus luteum [53]. The boost in fertility was assumed to be attributable to the intracellular antioxidant capabilities of Se, which protects cell membranes from damage caused by oxidation by removing “reactive oxygen species (ROS)” [54]. Ovulation, egg development, corpus luteum formation, embryo embedding, and embryo development all rely on ROS [55].

Serum analysis demonstrated an increase in progesterone and prolactin levels, showing that Se administration boosts antioxidant activity and combats oxidative stress, which improves reproductive efficiency [55]. Se supplementation enhanced the number and diameter of follicles, emphasizing its function in improving reproductive performance and follicle formation [56- 58].

Conclusion

In conclusion, according to the present research, it can be assumed that therapy with DFS hurts the female reproductive system by disrupting hormone concentrations and control. Se deficiency is an extremely prevalent problem worldwide. Supplementation is conceivable, but because Se has a tight safety margin, hazardous amounts are close to what is generally necessary for a proper requirement. Current findings might have been because the inducing bio-disposition enzymes, including Se, and improving systemic antioxidant state, may minimize DFS-associated cytotoxicity. Se regulates the manufacture of the active hormone T3 in the thyroid gland and surrounding tissues, which is critical for development and metabolism, it also performs an important involvement in growth and metabolism by regulating the synthesis of the active hormone T3 in the thyroid gland and surrounding tissues, while potentially protecting the female reproductive system by disrupting hormone concentrations caused by DFS and regulations.

Conflicts of Interest

The authors declared no competing interests of interest.

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