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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

Does celecoxib, a COX-2 inhibitor, spare the traditional nonsteroidal anti-inflammatory-mediated adverse events?

OBJECTIVE **To investigate the gastrointestinal (GI) and reproductive risk associated with chronic usage of celecoxib in male albino rats.** METHOD **Thirty male albino rats weighing 200–230 g were randomly divided into three equal groups of 10 rats each. In group 1, the control group, rats received no drug. In group 2, rats received celecoxib (50 mg/kg/day, orally) for 30 successive days. In group 3, rats received celecoxib (50 mg/kg/day, orally), and royal jelly (300 mg/kg/day, orally) for 30 successive days. Gastric ulcer scoring and semen analysis were employed to elucidate the celecoxib adverse effects on gastric and testicular tissue functions.** RESULTS **Celecoxib produced no gastric ulcer in both groups 2 and 3. Celecoxib has no appreciable effect on steroidogenesis (testosterone biosynthesis in Leydig cells of the testis) or spermatogenesis (sperms production from germ cells in the seminiferous tubules of the testis), as indicated by normal sperm count, motility, abnormalities, and normal serum testosterone levels. Royal jelly co-administration did not cause any significant change in serum testosterone level, sperm count, and sperm motility percentage, but caused a significant decline in sperm abnormalities level.** CONCLUSIONS **Upper GI tolerability and reproductive safety were proved in the chronic use of celecoxib in male albino rats, with ameliorative effects of royal jelly against celecoxib-induced oxidative and apoptotic stress.**

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ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2024, 41(6):771–782

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Με τη χρήση σελεκοξίμπης, ειδικού αναστολέα της COX-2, αποφεύγονται οι ανεπιθύμητες ενέργειες που προκαλούνται συνήθως από τα μη συνταγογραφούμενα αντιφλεγμονώδη φάρμακα;

Περίληψη στο τέλος του άρθρου

Key words

Celecoxib
Gastrointestinal
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Sperm
Testis
Testosterone

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The Food and Drug Administration (FDA) has given the drug family known as non-steroidal anti-inflammatory drugs (NSAIDs) approval for use as analgesic, antipyretic, and anti-inflammatory medications.[1] NSAIDs are often categorized into non-selective cyclooxygenase (COX) inhibitors (such as diclofenac, ibuprofen, indomethacin, ketoprofen, etc.) and selective COX-2 inhibitors (such as celecoxib), depending on their chemical structure and selectivity.[2]

The epidemiological and clinical literature has extensively covered the gastrointestinal (GI) adverse effects linked to the use of non-steroidal anti-inflammatory drugs. The COX enzyme is inhibited by NSAIDs, which are thought to harm the gastro-duodenum by inhibiting the COX-1 isoform. Celecoxib, a COX-2 selective inhibitor, has been demonstrated to be related to decreased gastroduodenal ulcer rates when compared to standard NSAIDs.[3] With greater GI tolerance than traditional NSAIDs, celecoxib may maintain similar analgesic and anti-inflammatory activity while being a better alternative for patients at risk of GI bleeding.[4] This is evidenced by lower incidence rates of gastric, duodenal, and gastroduodenal ulcers.[3]

It has been established that COX-2 is a key player in the negative autoregulation of steroidogenesis, presumably through the synthesis of PGE2 and PGF2.[5] Due to COX-2 suppression in the adult rat testis, celecoxib has been demonstrated in several studies to have direct or indirect effects on spermatogenesis.[6,7] Celecoxib taken at high doses has been shown to decrease the size and number of Leydig cells, which lowers testosterone levels.[8] As a result, prolonged high celecoxib doses are linked to male infertility.[9] Conversely, according to other studies, celecoxib has no discernible impact on steroidogenesis or

spermatogenesis, as evidenced by normal testicular weight, testicular morphology, or serum testosterone levels,[10] as well as normal sperm concentration, motility, and DNA fragmentation.[11,12] Men with normal spermatogenesis may benefit from being advised to take chronic celecoxib, but subfertile or infertile men should not.[13]

Royal jelly (RJ) is a nutrient-rich source of bioactive compounds that are essential to many biological processes. It has been determined that proteins, peptides, lipids, phenolics, and flavonoids are the main bioactive compounds responsible for the variety of pharmacological properties of RJ.[14] The use of RJ in the treatment of eye disorders, GI tract diseases, neurological disorders, fertility disorders, and wound healing activity was advised by its potential health benefits, such as antimicrobial, antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, cardioprotective, and hepatorenal-protective properties.[15]

Therefore, we studied the safety issue of celecoxib towards gastric ulcers and male fertility in male albino rats with special concern for the potential effect of RJ administration.

MATERIAL AND METHOD

Experimental design

Thirty, apparently healthy, male albino rats weighing 200–230 g were employed in this study. They were obtained from the laboratory animal farm, Faculty of Veterinary Medicine, Zagazig University. They were contained in clean cages with wire-bottomed galvanised metal walls. Throughout the experiment, they were given a balanced diet and unlimited access to fresh tap water. Before being enrolled in the study, they had a 7-day acclimatisation period. Rats were randomly divided into three equal groups; each of 10 rats: (a) Group 1 (control group): Rats in this group received no drug. Rats of this group served as the control group. (b) Group 2 (celecoxib group): Rats in this group received celecoxib (50 mg/kg/day, orally)[16] for 30 successive days, then rats were sacrificed and samples were collected. (c) Group 3 (celecoxib plus RJ group): Rats in this group received celecoxib (50 mg/kg/day, orally),[16] and RJ (300 mg/kg/day, orally)[17] for 30 successive days, then rats were sacrificed and samples were collected.

Samples collection and preservation

Blood samples

Blood samples were collected using a 3 mL syringe directly from the ventricular puncture of rats into centrifuge tubes and left to clot for 15 min at room temperature then centrifuged at 3,000 rpm for 10 minutes to allow serum separation, which was then aspirated into cryovials and stored at -20 °C for serum biochemical assays.

Tissue specimens

The liver, kidneys, heart, stomach, and testes tissues of each animal were dissected and collected as follows: (a) Gastric ulcer scoring: The stomach was quickly washed in an ice-cold saline solution (0.9% NaCL), containing 0.16 mg/mL heparin to remove red blood cells and a dissecting microscope with a square-grid eyepiece was used to quantify rats' stomach ulceration levels.[18]

Semen collection: One testis' cauda epididymis was quickly taken out and put in a sterile Petri plate with 2 mL of sodium chloride 0.9% solution at 37 °C. The epididymal contents were then extracted into the solution to create a suspension using a sterile scissor for semen analysis.[19]

Biological analysis

About 30 mg of the rat tissue samples were immediately collected, transferred in liquid nitrogen, and kept at -80 °C for total RNA extraction used for the determination of apoptotic and anti-apoptotic mRNA expression levels using RT-PCR,[20] for detection of apoptotic genes (*Bax*) and anti-apoptotic gene (*Bcl-2*).[21]

Biochemical measurements in sera

a) *Fertility tests. Measurement of serum testosterone concentration:* Quantitative estimation of testosterone (TH) concentration in serum was carried out using a commercially available Abnova® ELISA kit, according to the method of Granoff and Abraham.[22]

b) *Oxidant/antioxidant status:* Commercially available kits supplied from Oxi Select™, USA, were used for biochemical quantitative estimation of serum malondialdehyde (MDA), and superoxide dismutase (SOD), according to the method of Wang et al.[23]

Quantification of gastric ulceration

Rats' stomach ulceration levels were measured using the Szabo and Hollander technique.[18] In brief, stomachs that had been cleaned were pinned to a corkboard, and ulcers were graded on a 0–5 scale (depicting the severity of vascular congestions and lesions/hemorrhagic erosions). The ulcers were scored as follows: Almost normal mucosa: 0; vascular congestions: 1; one or two lesions: 2; severe lesions: 3; very severe lesions: 4; perforation: 5.

Mucosal damage was indicated as a percentage of the glandular stomach's overall surface area, which was calculated in square millimeters. Each rat's mean ulcer score was expressed as ulcer index (UI) and the percentage of inhibition against ulceration was determined using the expressions:

$$UI=[Ulcerated\ area/total\ stomach\ area]\times 100$$

$$\%Ulcer\ inhibition=[UI\ in\ control-UI\ in\ test]\times 100/UI\ in\ control$$

Semen analysis

The rat epididymal contents were extracted into 2 mL of sodium

chloride 0.9% solution in a sterilized Petri dish at 37 °C making semen suspension. Under a 40× high-power light microscope, sperm motility was analyzed. The sperm count was conducted at 100× magnification under a light microscope. The following formula was used to determine how many sperms were present in 1 mL of the fluid:

Mean number of sperm in each chamber×10,000×dilution factor

A microscope was used to assess morphological changes at 400× magnification. To measure the proportion of abnormal spermatozoa, about 100 spermatozoa were randomly observed in several fields while submerged in oil under an oil immersion lens. The following sperm traits were utilized to categorize them as abnormal: bent neck, bent tail, detached head, coiled tail, double head, amorphous head, curved tail, fused, looped tail, looped neck, detached tail, and hookless sperms.[24]

Apoptotic and anti-apoptotic genes expression

TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat. # P725, Enzynomics, Korea) was used for quantitative estimation of apoptotic genes *(Bax)* and anti-apoptotic gene *(Bcl-2)* based on the manufacturer's protocol.

Gene expressions were measured using the below formula and Ct (2-ΔΔCt) (fold change) method Eleawa et al.[20]

$$\Delta\Delta Ct=(Ct_{target}-Ct_{reference})\ test\ sample-(Ct_{target}-Ct_{reference})\ control\ sample$$

Finally, considering the primer efficiency value of approximately 2, the gene expression level was determined as 1-ΔΔCt.

Statistical analysis

The obtained data were analyzed statistically using the Statistical Package for Social Sciences (SPSS) program (version 26.0; SPSS Inc, IL, USA) for Microsoft Windows®. Results were expressed as the mean±standard deviation. One-way analysis of variance (ANOVA) was used to compare the means of the multiple groups in the study. Tukey HSD *post hoc* analysis was used for in-between group comparisons, where a p-value of less than 0.05 is considered to be significant.

RESULTS

Quantification of gastric ulceration

No gastric ulceration was detected in any of the study groups (fig. 1).

Fertility profile

Chronic celecoxib administration has no appreciable effect on testosterone hormone levels or spermatogenesis (sperms production from germ cells in the seminiferous tubules of the testis) as indicated by normal serum testosterone levels, normal sperm count, motility, and abnormalities. Royal jelly co-administration did not cause any significant change in serum testosterone level, sperm count, and motility percentage, but caused a significant decline in sperm abnormalities level (tab. 1).

Oxidant/anti-oxidant status

Chronic celecoxib administration impaired oxidant/antioxidant status as indicated by significant elevation ($p<0.05$) in serum MDA level, and significant decline ($p<0.05$) in serum SOD level. Royal jelly co-administration has a positive effect on oxidative status as indicated by a significant decline ($p<0.05$) in serum MDA level, and significant elevation ($p<0.05$) in serum SOD level (tab. 2).

Apoptotic and anti-apoptotic genes expression

Chronic celecoxib administration significantly upregulated ($p<0.05$) apoptotic gene (*Bax*/Gapdh %) expression

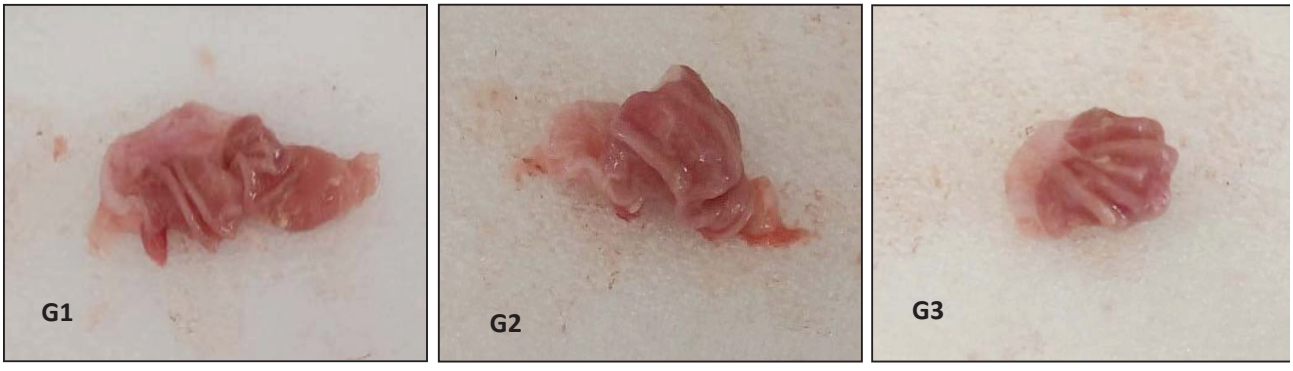

Figure 1. Effects of celecoxib (50 mg/kg/day, orally), royal jelly co-administration (300 mg/kg/day, orally), on gastric mucosa: No gastric ulcers. **Group 1 (G1)** received no drug (control). **Group 2 (G2)** received celecoxib (50 mg/kg/day, orally) for 30 successive days. **Group 3 (G3)** received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days.

and significantly downregulated (p<0.05) anti-apoptotic gene (*Bcl-2*/Gapdh %) expression levels in the stomach, and testis. Royal jelly co-administration significantly downregulated (p<0.05) apoptotic gene (*Bax*/Gapdh %) expression and significantly upregulated (p<0.05) anti-apoptotic gene (*Bcl-2*/Gapdh %) expression levels in the stomach, and testis (tab. 3).

Table 1. Effects of celecoxib (50 mg/kg/day, orally), and royal jelly (300 mg/kg/day, orally) on fertility profile in male albino rats. Data are expressed as mean±SD, n=10 per group.

Groups	Fertility profile			
	Testosterone (ng/mL)	Sperm motility (%)	Sperm count ($\times 10^6$)	Sperm abnormality (%)
1 (n=10)	3.30±1.85*	82.00±5.00*	93.33±37.17*	14.33±5.13*,**
2 (n=10)	2.60±0.65*	80.63±2.89*	89.00±7.81*	18.33±2.31*
3 (n=10)	2.90±0.40*	81.00±5.00*	90.33±4.16*	14.67±6.51*,**

Group 1 received no drug (control)

Group 2 received celecoxib (50 mg/kg/day, orally) for 30 successive days

Group 3 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days

Means carrying different superscripts in the same column are significant at p<0.05

SD: Standard deviation

Table 2. Effects of celecoxib (50 mg/kg/day, orally), and royal jelly (300 mg/kg/day, orally) on serum oxidative status profile in male albino rats. Data are expressed as mean±SD, n=10 per group.

Groups	MDA (nmoL/mL)	SOD (U/mL)
1 (n=10)	5.86±0.66***	5.40±0.62*
2 (n=10)	28.10±3.48*	0.89±0.68***
3 (n=10)	16.03±2.51**	3.20±0.61**

Group 1 received no drug (control)

Group 2 received celecoxib (50 mg/kg/day, orally) for 30 successive days

Group 3 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days

Means carrying different superscripts in the same column are significant at p<0.05

SD: Standard deviation, MDA: Malondialdehyde, SOD: Superoxide dismutase

DISCUSSION

Celecoxib is a NSAID used to treat mild to moderate pain and help relieve symptoms of various forms of arthritis and the management of acute or chronic pain due to its favorable GI toxicity profile.[25] Data indicate that celecoxib has no appreciable effect on steroidogenesis or spermatogenesis, at least in the short term, but may have implications for men with marginal fertility taking celecoxib for extended periods, especially in case of subfertility.[10] Celecoxib treatment mitigates oxidative stress,[26] but induces apoptosis.[27] Fortunately, RJ can ameliorate NSAIDs side effects.[17]

In our study, celecoxib administration (50 mg/kg/day, orally) for 30 successive days did not cause gastric ulcers in male albino rats. Celecoxib, a COX-2 inhibitor, does not block the constitutive COX-1 enzyme,[28] which is responsible for GI tract prostaglandins expression, that ensures gastric mucosal safety,[29] whereas it blocks the inducible COX-2 expression that occurs in case of tissue injury.[30]

Two different approaches were pursued in the search of GI sparing NSAIDs: (a) Classical COX hypothesis, where only COX-1 is responsible for gastric stomach integrity, and (b) modified COX hypothesis, where both COX-1 and COX-2 are involved in the protection of gastric mucosa.

In the classical COX hypothesis, COX-1-derived prostaglandins are considered to exert housekeeping functions in the gastric mucosa essential for normal gastric physiology. Inhibition of prostaglandin formation leads to a decrease in mucus and bicarbonate secretion, reduces mucosal blood flow, and causes vascular injury, leukocyte accumulation, and reduced cell turnover, all factors that contribute to the genesis of mucosal damage.[31] Selective COX-2 inhibitors do

Table 3. Effects of celecoxib (50 mg/kg/day, orally), and royal jelly (300 mg/kg/day, orally) on apoptotic and anti-apoptotic genes expression in male albino rats. Data are expressed as mean±SD, n=10 per group.

Groups	Apoptotic gene (Bax/Gapdh %)		Anti-apoptotic gene (Bcl-2/Gapdh %)	
	Stomach	Testis	Stomach	Testis
1 (n=10)	1.00±0.00***	1.00±0.00***	1.00±0.02*	1.00±0.00*
2 (n=10)	5.74±1.03*	6.16±1.3*	0.19±0.06***	0.30±0.03***
3 (n=10)	1.90±0.19**,***	2.35±0.18**,***	0.57±0.00*,**	0.81±0.12*,**

Group 1 received no drug (control)

Group 2 received celecoxib (50 mg/kg/day, orally) for 30 successive days

Group 3 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days

Means carrying different superscripts in the same column are significant at p<0.05

SD: Standard deviation

not affect COX-1 in the GI tract mucosa. They appear to be well tolerated by experimental animals and humans following acute and chronic (three or more months) administration. Thus, selective COX-2 inhibitors have been developed as GI-sparing anti-inflammatory drugs.[32,33]

The modern, modified COX hypothesis, has accused its predecessor of being oversimplistic.[34] It assumed that COX-1 and low levels of COX-2 contribute to gastric mucosal integrity. COX-1-derived prostaglandins regulate the mucosal blood flow and epithelial secretion of mucus and bicarbonate, while COX-2-derived prostaglandins affect epithelial proliferation and endothelial-leukocyte adherence,[35] which plays an important role in the healing of gastric ulcers.[34] Recent studies in rats have shown that neither selective COX-1 inhibition nor selective COX-2 inhibition is ulcerogenic individually.[36] Only combined inhibition of both COX-1 and COX-2 induces severe lesions in the stomach and small intestine, suggesting that reduction of mucosal blood flow (COX-1 inhibitors) and increase in leukocyte adhesion (COX-2 inhibitors) have to occur simultaneously to interfere with gastric mucosal defense.[37,38] These findings are confirmed by experiments using COX-1- or COX-2-deficient mice. Neither COX-1-knockout mice,[39] nor COX-2-deficient mice showed spontaneous GI lesions or even gastric pathology.[40]

Another advantage of celecoxib has been shown not to interfere with platelet function because it does not inhibit platelet thromboxane A_2 formation, which could be a contributory factor in the reduced incidence of GI hemorrhage.[41] Another not widely recognized advantage is its lack of interference with mitochondrial oxidative phosphorylation responsible for normal mucosal prostanoid levels important to gastric mucosa integrity.[42]

In addition to prostaglandins, other mediator systems, including nitric oxide (NO) have the potential to maintain gastric mucosal integrity in case of inhibition of prostaglandin synthesis[43] via inhibition of leukocyte adhesion to the endothelium, and healing of NSAID-induced damage.[44] Celecoxib beneficially lowers the expression of COX-2, hence reducing oxidative stress and increasing the bioavailability of NO.[45] Neither the COX-1 inhibitor (SC-560) nor COX-2 inhibitors (NS-398 or DFU) individually could cause stomach ulcers in the Ehrlich et al study. However, severe and dose-dependent injury developed when simultaneously the NO synthase was suppressed. Gastric mucosal damage induced by COX-2 inhibitors was only found after pre-treatment with a NO synthase inhibitor (L-NAME).[46] Celecoxib produced no gastric ulcer as it has been developed with several hundred-fold higher selectivity for COX-2 with no effect on COX-1 prostaglandin production responsible for gastric mucosa integrity.[47]

In accordance with our findings, studies have confirmed a better GI profile of celecoxib.[48,49] The incidence of GI ulcer complications associated with celecoxib was 8-fold lower than with nonspecific NSAIDs.[50] In patients at high risk of GI events who require concomitant aspirin and NSAID, celecoxib plus proton-pump inhibitor is the preferred treatment to reduce the risk of recurrent upper GI bleeding.[51] Treatment of gastric cancer with celecoxib is not associated with an increased risk of gastroduodenal ulcer.[52,53] Gastrointestinal tolerability of celecoxib in elderly patients aged 65 years or older with osteoarthritis, rheumatoid arthritis, or ankylosing spondylitis was higher in celecoxib than in conventional nonselective NSAIDs.[54] Celecoxib was found to be more effective in post-endodontic pain with minimal gastric symptoms, when compared to other NSAIDs.[55]

Without a doubt, RJ has a beneficial effect in preventing stomach ulcers. It inhibits inducible nitric oxide (iNOS) and nuclear factor kappa beta (Nf-κβ) activity in the gastric mucosa and prevents epithelial cell apoptosis. It also significantly decreases the activity of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1 beta (IL-1β) levels. In addition, a decrease in the myeloperoxidase (MPO) level with favorable tissue oxidative status indicated that RJ prevented tissue damage.[56]

In our study, celecoxib administration (50 mg/kg/day, orally) for 30 successive days was claimed to cause a non-significant decline in serum testosterone hormone level, sperm motility, and sperm count in male albino rats; in addition to non-significant elevation in sperm abnormalities percentage. An ameliorative effect of RJ against celecoxib-induced infertility.

In accordance with our findings, celecoxib, as a COX-2 inhibitor, has not uncovered obvious effects on normal fertility or testicular function.[10] To comprehend this hypothesis, we must be aware that both COX isoforms, referred to as COX-1 and COX-2, are expressed constitutively in male rat testes,[57,58] knowing that COX-2 is predominant.[59] The localization and expression levels of COX-2 significantly differ between the species. In rat testis, COX-2 has been found to localize in germ and Leydig cells, while in mice it is restricted to interstitial cells of Leydig cells.[60] In humans, discordant data are reported. While Frungieri et al confirmed the absence of COX-2 in male testes indicating impaired spermatogenesis if present,[61] Perrotta et al confirmed that both COX-1 and COX-2 are expressed and present in humans with normal spermatogenesis and COX-2 levels

are markedly increased in inflamed tissues as in varicocele and diabetic human donor's sperm.[62]

Undoubtedly, COX-2 possesses an important role in male reproduction activity via PGs production, which has important roles in both physiological and pathological processes in the testes.[63] Endogenous production of PGE_2 and $PGF_{2\alpha}$ by the testis can be attributed to COX-2 rather than COX-1.[11] COX-2/PGs are expressed in the two key somatic cell types in the testis, Leydig and Sertoli cells, under the regulation of hormonal input (FSH, prolactin, and testosterone), as well as by IL1β for the production of steroidogenesis in Leydig cells and glucose uptake in Sertoli cells. Hence, such COX-2/PG system acts as a local modulator of testicular activity and consequently may regulate spermatogenic efficiency.[12,64]

Regarding celecoxib, studies indicate both positive and negative effects of celecoxib in the preservation of male fertility. Chronic celecoxib would be of little consequence for males with optimal fertility. Oral administration of celecoxib, for 5 weeks, increases testicular interstitial fluid (IF) formation in the testis as a result of an increase in either testicular blood flow, capillary pressure, or vascular permeability, consistent with inhibition of rat testicular vasculature PGs production, but has no appreciable effect on steroidogenesis (steroid hormones, including testosterone, biosynthesis from cholesterol in Leydig cells of the testis) or spermatogenesis (sperms production from germ cells in the seminiferous tubules of the testis). Normal testis weight, testis morphology, serum testosterone levels, and normal sperm concentration, motility, and DNA fragmentation are confirmed.[10,13,65] In addition, celecoxib does not appear to alter the ability of the testis to mount an inflammatory response but opposes the deleterious effects of inflammation on IF formation and testosterone production, so it has the potential to ameliorate testicular damage caused by systemic or local inflammation,[10] and some toxicants in different pathological conditions in conjunction with serum and testis inflammatory cytokines, oxidative stress, or genetic alterations.[66] In this context, celecoxib offers protection against reproductive abnormalities induced by atrazine in male rats,[67] and against varicocele-induced damage at testicular and sperm levels in male rats.[68,69]

In contrast, celecoxib's negative impact on fertility is frequently associated with males with pre-existing subfertility or infertility. Increased testicular IF accumulation in the testis with increased capillary pressure causes inhibition of rat testicular vasculature PGs production and inhibition of T-type Ca^{2+} channels in spermatogenic cells which affects the Ca^{2+}, pH, and membrane potential homeostasis of sperm cells.[10,70]

The effect of RJ on sperm parameters and testosterone levels was studied extensively. The results were consistent with what our study found. Royal jelly (200 mg/kg, 400 mg/kg, and 800 mg/kg daily for 4 weeks, orally) in male Sprague-Dawley rats did not cause any significant change in sperm count because of all doses. No significant change in serum testosterone level or sperm deformity rate except for the high-dose group. High-dose RJ oral administration for 4 weeks adversely affected the reproductive system, in the form of an increase in the levels of estrogen, LH, FSH, and a decrease in testosterone level, which returned to normal levels after 14 days of RJ cessation.[71] Royal jelly (100 mg/kg daily for 48 days, orally) in male Wistar rats caused no change in sperm count, viability, motility, or testosterone production.[72] Royal jelly (100 mg/kg daily for 30 days, orally) in male mice caused a non-significant increase in sperm count, motility, and plasma testosterone levels.[73] Testosterone levels were non-significantly improved in men who received short-term RJ supplement (1,000 mg/daily/15 days),[74] long-term RJ supplement (100 mg daily for two months),[75] and increasing long-term RJ supplement (25 mg/kg, 50 mg/kg, and 100 mg daily for three months).[77] Interestingly, no significant difference in sperm count before and after consumption of RJ in all of the above investigations is reported.[74–76] Fortunately, RJ (100 mg/kg orally) significantly reduced the sperm abnormalities percentage in optimal[77] and high ambient temperature[78] conditions.

In the present study, celecoxib administration (50 mg/kg/day, orally) for 30 successive days was claimed to cause oxidative stress (OS) in male albino rats as manifested by significant elevation of serum malondialdehyde (MDA) and significant decline in superoxide dismutase (SOD) levels, with ameliorative effects of RJ against celecoxib-induced oxidative stress.

OS is a shift in the balance between the production rate of oxidants and their elimination via the antioxidant defense system.[79] OS-mediated molecules are an array of metabolites derived from molecular reactive oxygen species (ROS) and reactive nitrogen species (RNS).[80] Prime sites of ROS production in living organisms are the mitochondria, paroxysms, and endoplasmic reticulum. ROS are produced during the mitochondrial electron transport chain, through the oxidative phosphorylation pathway for ATP production.[81] ROS are also produced in phagocytes, auto-oxidation reactions, and subsequent to antioxidant enzymes (e.g., xanthine oxidase).[82] RNS is derived from nitric oxide (•NO) metabolism.[83] ROS/RNS are produced from either endogenous or exogenous sources. Immune system activation, inflammation, mental

stress, excessive exercise, cancer, infection, ischemia, and aging represent endogenous stressors, while air pollution, water pollution, alcohol, cigarette smoke, heavy metals, pharmaceutical agents, and ultraviolet radiation act as exogenous sources of OS.[84]

Antioxidants are the primary line of the body's defense system against oxidative stress. In physiological conditions, antioxidants inhibit the oxidation reaction of molecules that can produce free radicals.[85] Several molecules play a role in such defense, either internally synthesized (endogenous), or externally supplied through food (exogenous) antioxidants. Exogenous antioxidants (RJ for instance) act as direct ROS scavengers and increase the antioxidant enzyme activities.[86] Endogenous antioxidant defenses involve a network of antioxidant enzymatic and non-enzymatic molecules that are usually disseminated within the cytoplasm and various cell organelles.[87] Primary antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and several peroxidases, catalyze a complex cascade of reactions to transform ROS into more stable molecules, such as water and O_2. MDA is the end-product of polyunsaturated fatty acids oxidation in cellular membranes; thus, it acts as a dependable marker of oxidative stress.[88] Non-enzymatic antioxidants include vitamins (A, C, E, and K), enzyme cofactors (Q10), and minerals (Zn, Mn, Cu, Se, etc.).[89]

Following our findings, previous studies indicated an alteration in the oxidant/antioxidant status of rats treated with celecoxib.[90–93] The excessive generation of free radicals is considered one of the mechanisms by which celecoxib induces toxicity. Free radicals trigger the development of many diseases and cause harmful effects that cause the peroxidation of biomembranes and DNA, which is the reason for tissue destruction.[94]

Previous studies confirmed the concept of the beneficial and antioxidant effects of RJ.[95–97] Administration of RJ *in vitro* inhibited the production of pro-inflammatory cytokines, such as TNF-α and IL-1, IL-6 in a dose-dependent manner.[98] Other *in vivo* and *in vitro* studies also proposed anti-inflammatory and anti-oxidant properties of RJ.[99] The antioxidant activity of RJ can be attributed to the free radical scavengers; they can inhibit lipid peroxidation[100] via scavenging hydroxyl radicals,[101] and they attributed this activity to three dipeptides containing tyrosine residues. Additionally, RJ inhibits the enzymes that elevate the peroxidation of endogenous lipids, as well as cytochrome P450 expression, which is one of the cellular sources of oxygen radicals.[102]

In the present study, celecoxib administration (50 mg/kg/day, orally), for 30 successive days was claimed to induce an apoptotic effect in male albino rats as manifested by a significant elevation of serum apoptotic gene (*Bax*) and a significant decline in serum anti-apoptotic gene (*Bcl-2*) in stomach, and testis, with ameliorative effects of RJ against the celecoxib-induced apoptotic effect.

Apoptosis is programmed cell death, which involves the genetically determined elimination of cells. Since too little or too much cell death can result in pathology, such as autoimmune illnesses, neurodegeneration, or cancer, it is obvious that apoptosis needs to be strictly gene-regulated.[103] Several factors contributed to the apoptotic mechanism, among them, two main families of proteins including caspases and the *Bcl-2* family. Caspases, death-driving cysteine proteases, play an effective role in the apoptotic process. Activation of caspases ensures that the cellular components are degraded in a controlled manner, carrying out cell death with minimal effect on surrounding tissues.[104] Whereas, *Bcl-2*, consists of anti-apoptotic and pro-apoptotic members. The anti-apoptotic members of this family, such as *Bcl-2* and *Bcl-XL*, prevent apoptosis either by sequestering caspases or by preventing the release of mitochondrial apoptogenic factors, such as cytochrome c and apoptosis-inducing factor (AIF) into the cytoplasm, which are responsible for caspase activation. In contrast, pro-apoptotic members of this family, such as Bax and Bak, trigger the release of caspases by inducing the release of mitochondrial apoptogenic factors into the cytoplasm leading to caspase activation.[105]

Apoptosis is an essential physiological process for the selective elimination of individual cells without destruction or damage to the whole organ.[106] The morphological features of apoptosis include cell shrinkage and pyknosis, cytoplasmic and nuclear condensation, chromatin cleavage, apoptotic bodies formation, an intact plasma membrane, and exposure of surface molecules to phagocytosis.[107] In contrast, necrosis has been characterized as passive, accidental cell death that is triggered by external factors or disease with the uncontrolled release of inflammatory cells.[108,109] The main morphological changes associated with necrosis include cell swelling, cytoplasmic vacuole formation, distended endoplasmic reticulum, cytoplasmic bleb formation, condensed, swollen, or ruptured mitochondria, disrupted organelle membranes, swollen and ruptured lysosomes, and ultimately disruption of the cell membrane.[110] While necrosis is always pathological to an organ,[103] apoptosis is a normal and physiological process to get rid of certain cells that have been damaged beyond repair. It is almost always normal and advantageous. Additionally, apoptosis happens as a protective process, such as in immunological responses or when

diseases or toxic chemicals destroy cells. Thus, it keeps normal tissue function.[111]

Following our findings, previous studies have confirmed celecoxib-induced apoptosis. Celecoxib causes apoptosis by causing the loss of the mitochondrial transmembrane potential, the release of cytochrome c and AIF, and the activation of caspase-9 and caspase-3. Additionally, the anti-apoptotic protein Bcl-2 was reduced in abundance whereas the pro-apoptotic protein Bax was enhanced by celecoxib. The data showed that mitochondria-dependent signaling, not PPAR/NF-B signaling, was the mechanism through which celecoxib triggered apoptosis in mouse liver cancer cells.[27] In another study, celecoxib induced apoptosis in 5-fluorouracil-resistant gastric cancer cells through protein kinase B (PKB) inhibition,[112] which is a key component of the phosphatidyl-inositol-3 kinase (PI3K) intracellular pathway that exerts a pivotal role in regulating cell proliferation, survival, and metabolism.[113] Celecoxib induced apoptosis in glioblastoma tumor cells, the primary malignant tumor of the brain, via suppressing CIP2A/PP2A/Akt signaling axis.[114]

Several previous studies confirmed the concept of the beneficial and anti-apoptotic effect of RJ against cisplatin-induced hepatorenal toxicity,[115] nicotine-induced testicular injury in mice,[116] doxorubicin-induced nephrotoxicity in male albino rats,[117] and hydroxyurea-induced hepatic injury in rats.[118] Moreover, RJ decreases the expression of the apoptotic gene (*MMP-9*) responsible for bladder cancer in humans.

The limitation of our study was the relatively small final number of experimental animals in each group.

In conclusion, upper GI tolerability and reproductive safety were proved in the chronic use of celecoxib in male albino rats, with ameliorative effects of RJ against celecoxib-induced oxidative and apoptotic stress.

ΠΕΡΙΛΗΨΗ

Με τη χρήση σελεκοξίμπης, ειδικού αναστολέα της COX-2, αποφεύγονται οι ανεπιθύμητες ενέργειες που προκαλούνται συνήθως από τα μη συνταγογραφούμενα αντιφλεγμονώδη φάρμακα;

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ΣΚΟΠΟΣ Η διερεύνηση του γαστρεντερικού και του αναπαραγωγικού κινδύνου που σχετίζεται με τη χρόνια χρήση της σελεκοξίμπης (celecoxib) σε άρρενες αλμπίνο (albino) αρουραίους. **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Τριάντα αρσενικοί αλμπίνο αρουραίοι, βάρους 200–230 g, χωρίστηκαν τυχαία σε 3 ίσες ομάδες από 10 η κάθε μία: Ομάδα 1 (ομάδα ελέγχου): αρουραίοι που δεν έλαβαν φάρμακο, Ομάδα 2: αρουραίοι που έλαβαν σελεκοξίμπη (50 mg/kg/ημέρα, από το στόμα) για 30 διαδοχικές ημέρες και Ομάδα 3: αρουραίοι που έλαβαν σελεκοξίμπη (50 mg/kg/ημέρα, από το στόμα) και βασιλικό πολτό (300 mg/kg/ημέρα, από το στόμα) για 30 διαδοχικές ημέρες. Η βαθμολογία του γαστρικού έλκους και η ανάλυση σπέρματος χρησιμοποιήθηκαν για τη διερεύνηση των ανεπιθύμητων ενεργειών της σελεκοξίμπης στις λειτουργίες του γαστρικού ιστού και των όρχεων. **ΑΠΟΤΕΛΕΣΜΑΤΑ** Η σελεκοξίμπη δεν παρήγαγε γαστρικό έλκος και στις δύο ομάδες 2 και 3. Η σελεκοξίμπη δεν είχε αξιόλογη επίδραση στη στεροειδογένεση (βιοσύνθεση τεστοστερόνης στα κύτταρα Leydig του όρχεος) ή στη σπερματογένεση (παραγωγή σπέρματος από γεννητικά κύτταρα στα σπερματοζωάρια των όρχεων), όπως υποδείχθηκε από τον φυσιολογικό αριθμό σπερματοζωαρίων, την κινητικότητα, τις ανωμαλίες και τα φυσιολογικά επίπεδα τεστοστερόνης ορού. Η συγχορήγηση βασιλικού πολτού δεν συνοδεύτηκε από κάποια σημαντική αλλαγή στο επίπεδο τεστοστερόνης στον ορό, στον αριθμό των σπερματοζωαρίων και στο ποσοστό κινητικότητας των σπερματοζωαρίων, αλλά προκάλεσε σημαντική μείωση στο επίπεδο των ανωμαλιών του σπέρματος. **ΣΥΜΠΕΡΑΣΜΑΤΑ** Η ανεκτικότητα του ανώτερου γαστρεντερικού σωλήνα και η αναπαραγωγική ασφάλεια αποδείχθηκαν στη χρόνια χρήση της σελεκοξίμπης σε άρρενες αλμπίνο αρουραίους, με βελτιωτικά αποτελέσματα του βασιλικού πολτού έναντι του οξειδωτικού και αποπτωτικού stress που προκαλείται από τη σελεκοξίμπη.

Λέξεις ευρετηρίου: Βασιλικός πολτός, Γαστρεντερικό, Όρχεις, Σελεκοξίμπη (celecoxib), Σπέρμα, Τεστοστερόνη

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