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Relation of Antiulcer Effect of Progesterone Resulting from Follicle Stimulating Hormone Inhibition with Gastric Cyclooxygenase Levels in Female Rats

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Sex hormones have detrimental effects as well as beneficial ones. This study investigated effects of acute and chronic administration of progesterone and follicle stimulating hormone (FSH) on gastric ulcers and cyclooxygenase (COX) enzyme levels. Chronic progesterone at low doses (0.5 and 1.0 mg/kg), which did not stimulate PRs, reversed the reducing effect of FSH in gastric COX-1 level and decreased FSH-stimulated indomethacin ulcers by inhibiting FSH. Progesterone was observed to decrease COX-1 and increase ulcer area at dosages which stimulated PRs (3 mg/kg). FSH decreased COX-1 levels in gastric tissue *via* PRs. Mifepriston prevented the detrimental effects of progesterone and FSH in gastric tissue. Also inhibitory effects of 3 mg/kg progesterone on gastric COX-2 level were reversed by mifepriston. In conclusion low dose of chronic progesterone that inhibited FSH was beneficial in case of increasing gastroprotective COX-1 activity and healing indomethacin induced gastric ulcers.

Key Words: Cyclooxygenase, Follicle stimulating hormone, Ulcer, Progesterone, Rat.

INTRODUCTION

It is known that gastric ulcer is a multi-etiologic chronic disease. Various factors, such as the impairment of the balance between aggressive (increased acid secretions) and protective factors, helicobacter pylori, use of cigarettes and alcohol, steroid and non-steroid drugs, have been shown to play a role in gastric ulcer formation[1,2]. Non-steroidal antiinflammatory drugs (NSAIDs) are widely used in the treatment of pain, fever and inflammation. However, these drugs carry various side effects,

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especially those occurring in the gastrointestinal tract, such as gastric mucosal erosions, ulcerations, bleeding and perforations. There is also an increased risk of bleeding from preexisting peptic ulcers[3]. It is widely known, however, that NSAIDs produce beneficial effects through their ability to block cyclooxygenase (COX), thereby inhibiting prostaglandin (PG) production[4].

Cyclooxygenase enzymes catalyze the conversion of arachidonic acid to PGs and related eicosanoids. Two isoforms of the COX gene have been identified, which encode for constitutively expressed COX-1 and inducible COX-2[5]. COX-1 is known to enhance the physiologic integrity in thrombocytes, kidneys, gastrointestinal system (GIS) and endothelial cells. On the other hand, COX-2 is induced by cytokines, endotoxins and inflammatory mediators[6-8]. COX-1 functions as housekeeping enzyme responsible for the maintenance of homeostasis reactions. In particular, the formation of mucosaprotective prostaglandins in the gastrointestinal tract was attributed exclusively to the COX-1 isoform[9]. Induction of COX-2 was supposed to result in increased production of mediators that are involved in patho-physiological reactions such as inflammation[9]. Role of COX-2 expression in breast cancers has much evidence[10,11]. But it is also known that COX-2 is expressed under normal physiological conditions in the kidneys, uterus, vascular endothelium and GIS tissue[12]. It has been experimentally proven that COX-1 takes part in the synthesis of gastroprotective prostaglandins[6,13]. COX-1 and its derivatives (PGE2) protect GIS mucosa by decreasing acid secretion and increasing mucus secretion, by stimulating bicarbonate secretion and improving mucosal blood flow[14].

Progestins (progesterone) are female sex hormones secreted from the ovarium[15]. Follicle stimulating hormone (FSH) is a hormone secreted by the pituitary gland in the brain. Follicle stimulating hormone stimulates the follicles in the ovaries to ripen several eggs. Follicle stimulating hormone also readies the mammary glands for milk production. Progestins prepare the uterus for pregnancy and the breast glands for lactation[16]. These hormones were demonstrated to have detrimental effects as well as beneficial ones. It is also known that progestins cause these effects *via* their own receptors. Overproduction of endogenous progesterone or administration of this hormone externally has reduced gastric damage[17]. However, it has been found that progesterone is ulcerative in high doses and antiulcerative in FSH-inhibitory chronic low doses[18]. Many studies investigated relation between these hormones and cyclooxygenase enzymes in different cancer models, particularly in breast cancer[19]. But there is no study about their effect on stomach COX levels.

For these reasons, the aim of this study is to investigate gastro-protective and gastro-toxic effects of acute and chronic administration of progesterone and FSH and to determine the relationship between their effects and COX levels.

EXPERIMENTAL

A total of 126 female Albino Wistar rats weighing 190-210 g were obtained from the Ataturk University Medicinal and Experimental Application and Research

Center for use in this study. The animals were allocated to treatment groups prior to initiation of experimental procedures. They were housed and fed in the laboratory at 22 ± 0.1 ºC under standard conditions.

All chemicals for laboratory experimentation were purchased from Sigma Chemical Co. (Germany). Also, thiopental sodium, progesterone and FSH were purchased from SIGMA.

Effect of acute (single dose) administration of progesterone and FSH on indomethacin induced gastric ulcers in rats: Six groups of rats (n = 6) were fasted 24 h prior to experiment with free access to water. Progesterone (0.5, 1.0 and 3.0 mg/kg) was applied to defined groups of rats by oral gavage. Conversely, FSH (100 and 200 U/kg) was injected in other groups of rats intraperitoneally. Distilled water was given as a vehicle to a control group. 5 min after drug administration indomethacin (25 mg/kg) was administered to all rat groups by oral gavage. 6 h after drug administration, the rats were killed by an overdose of a general anesthetic (thiopental sodium, 50 mg/kg). The stomachs of the rats were removed and ulcer areas were determined on square millimeter paper[20]. Then the stomachs were transported to the biochemistry laboratory for measurement of COX-1 and COX-2 levels.

Effect of chronic administration of progesterone and FSH on indomethacin induced gastric ulcers in rats: In this series of experiments, progesterone and FSH were applied to rats at defined doses over a period of 10 days. At the ninth day of drug administration animals were fasted 24 h with free access to water. 5 min after the final dose of drug was administered, indomethacin (25 mg/kg) was administered to all rat groups by oral gavage. 6 h after indomethacin administration, the rats were killed by an overdose of a general anesthetic (thiopental sodium, 50 mg/kg). The stomachs of the rats were removed and ulcer areas were determined on square millimeter paper. Then the stomachs were transported to the biochemistry laboratory for measurement of COX-1 and COX-2 levels.

Effect of acute administration of progesterone and FSH on indomethacin induced gastric ulcers in mifepriston-treated rats: In this experiment, 5 groups of rats received a dose of mifepriston (50 mg/kg) intra-peritoneally. 30 min after mifepriston injection, the rat groups received progesterone (0.5, 1.0 and 3.0 mg/kg) and FSH (100 and 200 U/kg), respectively. The 6th group received distilled water as vehicle. 5 min after drug administration, indomethacin (25 mg/kg) was administered to all rat groups by oral gavage. 6 h after the hormone treatment the rats were killed by an overdose of a general anesthetic (thiopental sodium, 50 mg/kg). The stomachs of the rats were removed and ulcer areas were determined on square millimeter paper. Then the stomachs were transported to the biochemistry laboratory for measurement of COX-1 and COX-2 levels.

Biochemical analyses

Measurement of COX activity: At this series of present experiments, COX activity of rat' stomachs was measured *via* COX activity assay kit (Cayman, Ann

Arbor, MI, USA). Stomach tissue were collected free of stomach membranes and washed thoroughly with ice-cold Tris buffer, pH 7.4, containing 0.16 mg/mL of heparin to remove any red blood cells and clots, then stored at -80 ºC until assayed. A sample of stomach tissue was homogenized in 5 mL of cold buffer (0.1 M tris-HCl, pH 7.8, containing 1 mM EDTA)/g of tissue and centrifuged at 10,000 × g for 15 min at 4 ºC. Supernatant was removed for assay and stored on ice. Protein concentration in the supernatant was measured by the Bradford method[21]. The COX kit measures the peroxidase activity of COX. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-*p*-phenylenediamine at 590 nm. COX-2 activity was measured using the COX-1-specific inhibitor. Results are given as unit/milligram of protein for COX-1 and COX-2 activity.

Statistical analyses: Data of enzyme activity were subjected to one-way ANOVA using SPSS 13.0 software. Differences among groups were attained using the Tukey option and significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of acute (single dose) administration of progesterone and FSH on indomethacin induced gastric ulcers in rats: Table-1 shows acute administration of progesterone at 0.5, 1.0 and 3.0 mg/kg doses produced 20.5, 18.7 and 35.0 mm^2 ulcer area in stomach tissue of rats. The ulcer areas were determined as 37.2 and 39.5 mm^2 for 100 and 200 mg kg doses of FSH while that was 21.7 mm^2 in the control group that received indomethacin only.

TABLE-1
EFFECT OF ACUTE (SINGLE DOSE) ADMINISTRATION OF PROGESTERONE AND FSH ON INDOMETHACIN INDUCED GASTRIC ULCERS IN RATS. N: NUMBER OF ANIMALS

Drugs	Dose	N	Ulcer area	p
Progesterone	0.5 mg/kg	6	20.5 ± 2.88	> 0.05
Progesterone	1.0 mg/kg	6	18.7 ± 2.4	> 0.05
Progesterone	3.0 mg/kg	6	35.0 ± 3.0	< 0.01
FSH	100 U/kg	6	37.2 ± 2.6	< 0.01
FSH	200 U/kg	6	39.5 ± 2.1	< 0.01
Control (indomethacin)	25.0 mg/kg	6	21.7 ± 1.9	–

FSH = Follicle stimulating hormone

Effect of chronic administration of progesterone and FSH on indomethacin induced gastric ulcers in rats: In the stomach tissue of rat groups received chronic progesterone at 0.5, 1.0 and 3.0 mg/kg doses 24.7, 11.5 and 33.5 mm^2 ulcer areas occurred. The ulcer areas were determined as 48.1 and 1.7 mm^2 for 100 and 200 mg kg doses of FSH while that was 19.8 mm^2 in the control group that received indomethacin only (Table-2).

Effect of progesterone and FSH on indomethacin induced gastric ulcers in mifepriston-treated rats: The mean ulcer area in mifepriston + 0.5 mg/kg progest-

TABLE-2
EFFECT OF CHRONIC ADMINISTRATION OF PROGESTERONE AND FSH ON INDOMETHACIN INDUCED GASTRIC ULCERS IN RATS. N: NUMBER OF ANIMALS

Drugs	Dose	N	Ulcer area	p
Progesterone	0.5 mg/kg	6	24.7 ± 2.2	> 0.05
Progesterone	1.0 mg/kg	6	11.5 ± 1.9	< 0.05
Progesterone	3.0 mg/kg	6	33.5 ± 2.6	< 0.05
FSH	100 U/kg	6	48.2 ± 4.7	< 0.05
FSH	200 U/kg	6	51.7 ± 4.6	< 0.05
Control (Indomethacin)	25.0 mg/kg	6	19.8 ± 2.1	–

erone, 1 mg/kg mifepriston + progesterone and 3 mg/kg mifepriston + progesterone groups were 19.5, 20.3 and 21.2 mm^2. 100 and 200 mg/kg doses of FSH produced 21.2 and 21.8 mm^2 ulcer area, respectively in the rats which were pretreated with mifepriston. There was 22.5 mm^2 ulcer area in the control group that received indomethacin only (Table-3).

TABLE-3
EFFECT OF PROGESTERONE AND FSH ON INDOMETHACIN INDUCED GASTRIC ULCERS IN MIFEPRISTON-TREATED RATS. N: NUMBER OF ANIMALS

Drugs	Dose	N	Ulcer area	p
Progesterone + mifepriston	0.5 mg/kg + 50.0 mg/kg	6	19.5 ± 1.9	> 0.05
Progesterone + mifepriston	1.0 mg/kg + 50.0 mg/kg	6	20.3 ± 2.8	> 0.05
Progesterone + mifepriston	3.0 mg/kg + 50.0 mg/kg	6	21.2 ± 3.4	> 0.05
FSH + mifepriston	100 U/kg + 50.0 mg/kg	6	21.8 ± 2.3	> 0.05
FSH + mifepriston	200 U/kg + 50.0 mg/kg	6	23.0 ± 2.9	> 0.05
Control (indomethacin)	25.0 mg/kg	6	22.5 ± 2.9	–

Results of biochemical analyses

Effect of acute (single dose) administration of progesterone and FSH on gastric COX-1 and COX-2 levels in rats: As seen in Fig. 1, COX-1 and COX-2 levels were determined as 231.8 ± 10.2 ($p > 0.05$) and 142.0 ± 8.9 ($p > 0.05$), 228.8 ± 10.6 ($p > 0.05$) and 149.3 ± 5.6 ($p > 0.05$), 187.2 ± 6.0 ($p < 0.05$) and 115.0 ± 10.2 ($p < 0.05$) U/mg protein in the rat groups received 0.5, 1.0 and 3.0 mg/kg doses of progesterone, respectively. In the FSH groups (100 and 200 mg/kg) the COX-1 levels were 193.6 ± 9.1 ($p < 0.05$) and 185.3 ± 9.5 U/mg protein ($p < 0.05$) while COX-2 levels were 150.9 ± 15.4 ($p > 0.05$) and 157.0 ± 9.6 ($p > 0.05$) U/mg protein, respectively. The levels of COX-1 and COX-2 enzymes in control group which received indomethacin were 237.2 ± 16.1 and 155.5 ± 8.5 while that were 519.3 ± 19.6 ($p < 0.05$) and 205.0 ± 10.4 ($p < 0.05$) in the intact rat group which received no drug administration.

Effect of chronic administration of progesterone and FSH on gastric COX-1 and COX-2 levels in rats: COX-1 and COX-2 levels were determined as 235.5 ± 12.9 ($p > 0.05$) and 148.2 ± 6.4 ($p > 0.05$), 287.3 ± 10.8 ($p < 0.05$) and 144.2 ± 5.3

($p > 0.05$), 128.8 ± 8.6 ($p < 0.05$) and 110.7 ± 8.6 ($p < 0.05$) U/mg protein in the rat groups received 0.5, 1.0 and 3.0 mg/kg doses of progesterone, respectively. In the FSH groups (100 and 200 mg/kg) the COX-1 levels were 119.6 ± 6.1 ($p < 0.05$) and 115.5 ± 10.4 U/mg protein ($p < 0.05$) while COX-2 levels were 153.0 ± 10.2 ($p > 0.05$) and 158.0 ± 9.2 ($p > 0.05$) U/mg protein, respectively. The levels of COX-1 and COX-2 enzymes in control group which received indomethacin were 246.7 ± 11.6 and 150.3 ± 10.3 while that were 544.3 ± 14.1 ($p < 0.05$) and 225.0 ± 13.9 ($p < 0.05$) in the intact rat group (Fig. 2).

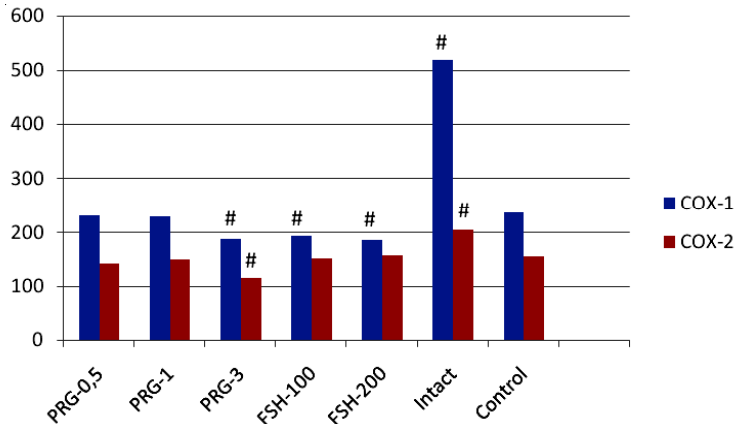

Fig. 1. Effect of acute (single dose) administration of progesterone (PRG) and FSH in gastric tissue of rats on COX-1 and COX-2 levels. #Significant at $p < 0.05$

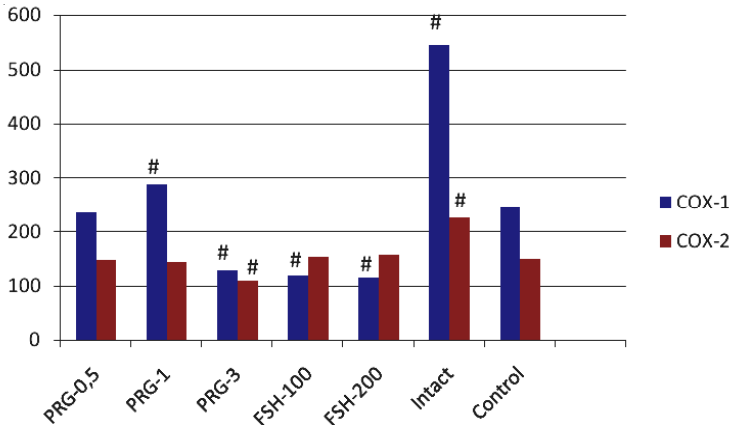

Fig. 2. Effect of chronic administration of progesterone (PRG) and FSH in gastric tissue of rats on COX-1 and COX-2 levels. #Significant at $p < 0.05$

Effect of progesterone and FSH on gastric COX-1 and COX-2 levels in mifepriston-treated rats: In mifepriston + 0.5 mg/kg progesterone, mifepriston+ 0.5 mg/kg progesterone, mifepriston+ 1 mg/kg progesterone and mifepriston+ 3 mg/kg progesterone groups

COX-1 and COX-2 levels were 225.7 ± 12.4 ($p > 0.05$) and 147.5 ± 8.9 ($p > 0.05$), 239.5 ± 11.0 ($p > 0.05$) and 157.2 ± 8.1 ($p > 0.05$), 234.4 ± 9.7 ($p > 0.05$) and 157.2 ± 8.1 ($p > 0.05$) U/mg protein, respectively. The levels of COX-1 and COX-2 enzyme in the groups received FSH at the doses of 100 and 200 mg/kg after 50 mg/kg dose of mifepriston pretreatment were 228.0 ± 12.2 ($p > 0.05$) and 153.0 ± 7.2 ($p > 0.05$), 223.0 ± 12.8 ($p > 0.05$) and 155.5 ± 6.4 ($p > 0.05$) U/mg protein, respectively. COX-1 and COX-2 levels were determined as 520.0 ± 16.1 and 221.7 ± 11.1 ($p > 0.05$), 225.5 ± 14.2 and 148.0 ± 9.4 in the intact rats and control rats, respectively (Fig. 3).

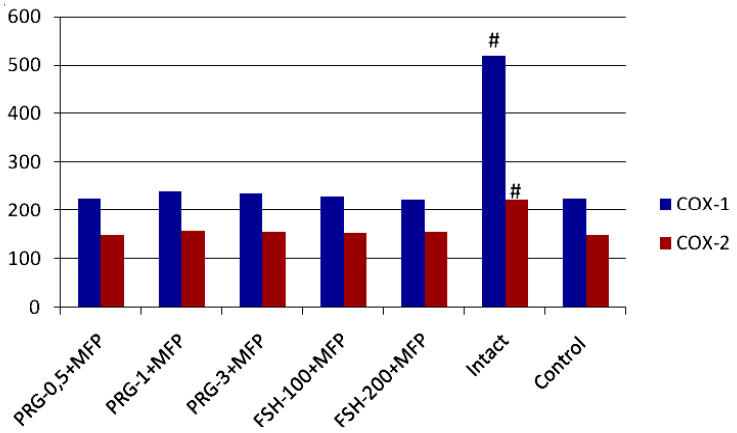

Fig. 3. Effect of progesterone and FSH on gastric COX-1 and COX-2 levels in mifepriston-treated rats. #Significant at $p < 0.05$

In this study, it was investigated whether gastro protective and gastro toxic effect of acute and chronic administration of progesterone and FSH had a relationship with cyclooxygenase enzyme levels or not.

In the first series of present experiments, it was observed that when progesterone is administrated at the acute lower doses (0.5 and 1.0 mg/kg), the progesterone receptor (PR) is not stimulated and no significant alteration occurred in indomethacin-induced ulcers. However, 3 mg/kg dose of progesterone can stimulate progesterone receptors, increased the ulcer index when compared to indomethacin group (control). Also in these experiments low doses of progesterone found to not affect on COX-1 level, while higher dose (3 mg/kg) decreased COX-1 levels significantly in stomach tissue when compared to control group. These data demonstrate that low doses of acute progesterone that can not stimulate progesterone receptors, have not an ulcerogenic effect while high doses of progesterone. The ulcerogenic effect is related to decreased levels of COX-1. The crucial roles of COX enzymes have been examined in gastric tissue in detail. COX-1 products, prostaglandins (PGI2 and PGE2) maintain integrity of gastrointestinal system (GIS) by reducing gastric acid secretion, increasing the thickness of mucus layer, stimulating bicarbonate secretion and enhancing mucosal blood flow[14,22-24]. PGE2 enhances mucus secretion by activating cAMP in

gastric epithelial cells[25]. Glucocorticoids and endogenous steroids can suppress the gene responsible for COX-2 synthesis[26,27]. Present results are in line with previous literature demonstrating different effects of low and high doses of progesterone in gastric ulcer[18]. In present study, 3 mg/kg dose of acute progesterone that can stimulate progesterone receptors decreased COX-2 levels significantly, but lower doses, which are not sufficient for receptor stimulation, failed to effect COX-2 levels. Progesterone is known to have a steroidal structure[15] which may be responsible for antiinflammatory effects. Nakagawa *et al.*[28] demonstrated antiinflammatory effects of progesterone. Then inhibition in COX-2 levels by progesterone administration may be related to its antiinflammatory effects. In the same series of present experiment both doses of acute FSH (100 and 200 U/kg) found to increase ulcer index and decrease COX-1 levels significantly in stomach tissue. Follicle stimulating hormone has also been reported to stimulate indomethacin induced gastric ulcers[18]. Borekci *et al.*[18] reported higher ulcer index in ovariectomized rats than intact rats and concluded that the increased indomethacin-induced ulcer incidence in the ovariectomized rat is related to progesterone deficiency, resulting in high concentrations of endogenous FSH. None of FSH doses we used affected COX-2 levels.

In the second series of present experiments chronic administration of 0.5 mg/kg progesterone produced no alteration in ulcer index, while 1 mg/kg dose decreased indomethacin induced ulcers significantly. Chronic administration of FSH at above mentioned doses and progesterone at the higher dose (3 mg/kg) which stimulates the PR, has demonstrated clear damage in gastric tissue. When we evaluated COX-1 levels, it is observed that the results of biochemical analyses are in line with our macroscopic ulcer scores. We suggest that 0.5 mg/kg dose of progesterone could not be enough for neither progesterone receptor stimulation nor FSH inhibition and so did alter neither ulcer index nor COX-1 levels. However 1 mg/kg dose of progesterone, which can not stimulate its own receptors, decreased ulcer index and increased COX-1 levels significantly. It is a fact that long-time (chronic) usage of progesterone inhibits FSH and luteinising hormone (LH) secretion[15]. We consider that antiulcer effect of chronic progesterone administration at 1 mg/kg dose is related to its inhibitory effect on FSH level as it has been previously reported[18]. Also luteinising hormone has been reported to be an antiulcerogenic agent[29] but inhibitory effects of 1 mg/kg progesterone on FSH may be stronger than that on luteinising hormone, so it may produce antiulcer effect *via* an FSH related pathway. Follicle stimulating hormone did not alter COX-2 levels at any dose we used but chronic administration of high dose progesterone decreased COX-2 level similar to acute administration.

In the last series of present experiments mifepriston was shown to inhibit the gastrotoxic effects of progesterone and FSH in gastric tissue. Also the alterations in COX-1 and COX-2 levels of progesterone and FSH groups have been prevented by mifepriston application. Mifepriston is known to be a PR antagonist[30]. Inhibition of gastrotoxic effects of progesterone and FSH *via* mifepriston demonstrates that these hormones affects gastrointestinal tract *via* progesterone receptors[18]. Present result

and literature review reveal that activation of progesterone receptor *via* high dose progesterone and FSH leads COX-1 reduction and blockage of these receptors *via* mifepriston application reversed the reduction in COX-1 levels. The reduction in COX-1 levels causes ulcer formation in progesterone and FSH groups. The combined application of mifepriston with progesterone and FSH also did not alter COX-2 levels. In many studies investigating COX levels in breast cancers; COX-2 levels were found to be excessively high. Increased COX-2 activity in cancers may be the result of inflammatory reactions occurred in cancer pathogenesis[31].

In conclusion, chronic administration of progesterone at a low dose (1 mg/kg), which did not stimulate progesterone receptors, reversed the reducing effect of FSH in gastric tissue COX-1 level by inhibiting FSH. Progesterone was observed to decrease COX-1 at dosages which stimulated progesterone receptors (3 mg/kg). Follicle stimulating hormone also decreased COX-1 levels in gastric tissue *via* progesterone receptors. In the light of these data it is concluded that low doses of progesterone that inhibited FSH were beneficial in case of increasing gastroprotective COX-1 activity. High doses of progesterone that can stimulate progesterone receptors and FSH decrease COX-1 activity *via* progesterone receptors resulting in gastric ulcer.

REFERENCES

1. W.A. Hooderwerf and P.J. Pasricha, in: L.L. Brunton (Ed.), Mc Graw-Hill, New-York, pp. 967-981 (2006).
2. F. Feldman and L.S. Friedman, WB Saunders Co., Philadelphia (2002).
3. J.L. Wallace, *Best Pract. Res. Clin. Gastroenterol*, **15**, 691 (2001).
4. J.R. Vane and R.M. Botting, *Int. J. Tissue React*., **20**, 3 (1998).
5. W.L. Smith and D.L. Dewitt, *Adv. Immunol*., **62**, 167 (1996).
6. J.G. Ferraz, K.A. Sharkey, B.K. Reuter, S. Asfaha, A.W. Tigley, M.L. Brown, W. McKnight and J.L. Wallace, *Gastroenterology*, **113**, 195 (1997).
7. J.A. Mitchell and T.D. Warner, *Br. J. Pharmacol*., **128**, 1121 (1999).
8. H. Suleyman, B. Demircan and Y. Karagoz, *Pharmacol. Rep.*, **59**, 247 (2007).
9. J.R. Vane and R.M. Botting, *Inflamm. Res*., **44**, 1 (1995).
10. E. Half, X.M. Tang, K. Gwyn, A. Sahin, K. Wathen and F.A. Sinicrope, *Cancer Res.*, **62**, 1676 (2002).
11. G.S. Ranger, A. Jewell, V. Thomas and K. Mokbel, *J. Surg. Oncol*., **88**, 100 (2004).
12. M. Katori and M. Majima, *Inflamm. Res*., **49**, 367 (2000).
13. L. Laine. *J. Pain Symptom. Manage*., **25**, S32 (2003).
14. C.J. Hawkey, *Gastroenterology*, **119**, 521 (2000).
15. D.S. Loose-Mitchell and G.M. Stancel, in: J.G. Hardman, L.E. Limbird and A.G. Gilman (Eds.), McGraw-Hill Companies New York, pp. 1597-1634 (2001).
16. L. Speroff and M.A. Fritz, Lippincott Williams and Wilkins., Philadelphia (2005).
17. C. Montoneri and F. Drago, *Dig. Dis. Sci*., **42**, 2572 (1997).
18. B. Borekci, E. Cadirci, A. Albayrak, H. Suleyman, Z. Halici and S. Kadanali, *Fertil. Steril.*, **90**, 1899 (2008).
19. G. Singh-Ranger, M. Salhab and K. Mokbel, *Breast Cancer Res. Treat*., **109**, 189 (2008).
20. H. Suleyman, F. Odabasoglu, A. Aslan, A. Cakir, Y. Karagoz, F. Gocer, M. Halici and Y. Bayir, *Phytomedicine*, **10**, 552 (2003).
21. M.M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).

22. P.C. Isakson, K.M. Verburg and C. Maziasz, *Gastroenterol. Int*., **12**, 169 (1999).
23. K. Takeuchi, S. Kagawa, H. Mimaki, M. Aoi and S. Kawauchi, *Dig. Dis. Sci*., **47**, 2116 (2002).
24. I. Tegeder, W. Neupert, H. Guhring and G. Geisslinger, *J. Pharmacol. Exp. Ther*., **292**, 1161 (2000).
25. S. Tani, T. Suzuki, S. Kano, T. Tanaka, K. Sunaga, R. Morishige and T. Tsuda, *Biol. Pharm. Bull*., **25**, 14 (2002).
26. J.L. Masferrer, K. Seibert, B. Zweifel and P. Needleman, *Proc. Natl. Acad. Sci. (USA)*, **89**, 3917 (1992).
27. J.A. Mitchell, M.G. Belvisi, P. Akarasereenont, R.A. Robbins, O.J. Kwon, J. Croxtall, P.J. Barnes and J.R. Vane, *Br. J. Pharmacol*., **113**, 1008 (1994).
28. H. Nakagawa, K.R. Min, K. Nanjo and S. Tsurufuji, *Jpn. J. Pharmacol*., **29**, 509 (1979).
29. B. Borekci, Y. Kumtepe, M. Karaca, Z. Halici, E. Cadirci, F. Albayrak, B. Polat and H. Suleyman, *Gynecol. Endocrinol*., **25**, 264 (2009).
30. I.M. Spitz, *Curr. Opin. Investig. Drugs*, **7**, 882 (2006).
31. L.R. Howe, *Breast Cancer Res*., **9**, 210 (2007).

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