

Effects of Gender on Stress Ulcer Formation in Rats

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USLU, E., AYDIN, S., CARKMAN, S., UZUN, H., ALTINLI, E., APAYDIN, B.B., MEMISOGLU, K. and ERGINOZ, E. *Effects of Gender on Stress Ulcer Formation in Rats.* Tohoku J. Exp. Med., 2002, 197(1), 17-26 — In the experimental stress literature, the results of investigations have not shown a specific sex-dependent vulnerability to stress ulceration. The aim of this study was to evaluate the importance of sex differences on stress ulcer development. Related to gender, the contributing factors for stress ulcer production such as luminal acidity, sialic acid as an marker of gastric mucosal protection, oxygen (O₂)-derived free radicals and endogenous antioxidant defence mechanisms were also investigated. Fifty Wistar Albino rats weighing about 230 g and aged 7 or 8 months were divided equally into five groups: Group I normal male rats, group II castrated male rats, group III normal female rats in estrus phase, group IV normal female rats in diestrus phase and group V castrated female rats. Cold restraint model was used for 6 hours to produce stress ulcer. No statistically significant difference was found out between groups in view of gross and histopathologic damage. There was no significant difference between groups according to gastric luminal acidity, gastric mucosal sialic acid, gastric malonaldehyde (MDA) and catalase values. Gastric superoxide dismutase (SOD) activity was significantly lower in Group I in comparison to those of Group III and IV. Sex differences do not interfere stress ulcer formation. SOD activity in rat gastric tissue has varied significantly by hormonal milieu. ——— gender; ulcer; sialic acid; free radical

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Acute gastric mucosal lesions, so called acute stress ulcer, is a common cause of gastro-

intestinal bleeding of the patients having treatment because of shock, sepsis, respiratory

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insufficiency and multiple trauma in intensive care units (Cheung 1988; Fenton et al. 1992).

The pathogenesis of the gastric stress ulcer is multifold. Intraluminal acid must be present for the formation of mucosal damage (Mersereau and Hinchey 1973). Current concepts suggest that gastric adherent mucus plays an important role in protecting the mucosa against ulceration and this protective force breaks down during stress ulcer formation as indicated by low level of its sialic acid component (Somasundaram and Ganguly 1985). Cellular injury by oxygen derived free radicals has been considered as a common pathway of tissue damage in several pathological processes (Fridovich 1978; Brown and Fridovich 1980). Recent studies reported that ischemia-induced oxygen derived free radicals are also responsible for the occurrence of gastric mucosal lesions (Perry et al. 1986; Andrews et al. 1992). Radical scavengers such as superoxide dismutase (SOD) have been shown to be effective in reducing the adverse effects of free radicals on gastric mucosa (Salim 1990; Itoh and Guth 1984; Ohara et al. 1991).

Results of the experimental stress ulcer studies varies from each other; some suggested male rats were more prone to develop stomach ulcer (Sawrey and Long 1962) whereas others reported female dominance (Lambert 1968; Herner and Caul 1972; Pare et al. 1978; Lambert and Kinsley 1993; Pare and Redei 1993)

The aim of this study was to evaluate the importance of gender in the development of stress ulcer. Our study differs from the other studies done in this field. Related to gender, the factors which are implicated to stress ulcer formation such as luminal acidity, gastric mucosal sialic acid, O₂-derived free radicals and endogenous antioxidant defence mechanism were also investigated.

MATERIALS AND METHODS

Study design

All experiments were done in accordance with the standards in "The UFAW Handbook on the Care and Management of Laboratory Animals" (Poole 1999).

In this study, 50 Wistar-Albino rats weighing about 230 g (210–240) and aged 7 or 8 months were used. The rats were divided into 5 groups each containing 10 rats. Group I; normal male rats, Group II; castrated male rats, Group III; normal female rats in estrus phase, Group IV; normal female rats in diestrus phase, and Group V; castrated female rats. The castration procedure consisting of bilateral orchietomy in male rats (Group II) and bilateral oophorectomy in female rats (Group V) was performed 7 days before the beginning of stress ulcer production. At the same day in groups I, III and IV sham operation was performed.

Experimental model

The experimental study was completed in five consecutive days. Rats were starved 12 hours before the experiment with free access of water. The estrus cycle of all female rats was determined by taking daily vaginal smear for 18 days. In each experimental study, at 08.00, two rats in each group were selected for restraint model previously described by Brodie and Hanson (1960) The rats were anesthetized lightly with ether and restrained on a rodent immobilization device spaced 15 cm apart from each other for 6 hours at 16°C. At the end of the restraint period, rats were sacrificed by prolonged ether anaesthesia. Laparotomy was done and the stomachs were dissected out. The excised stomach was placed on an ice bearing surface and was opened along the greater curvature.

Gastric mucosal pH measurement and gross examination

Gastric mucosal pH was measured with an

indicator paper (pH fix 0-14 Art N 92100, Machoney Nagel GmBh Duren, Germany). Thereafter the mucosal surface was gently rinsed by cold saline solution. Stomach was stretched over the ice. The mucosal lesions were inspected and rated for gross pathology according to the scale described by Dekansky et al. (1975): 0=no damage, 1=blood at the lumen, 2=pin point erosions, 3=1-5 small erosions < 2 mm, 4=>5 small erosions < 2 mm, 5=1-3 large erosions > 2 mm, 6=>3 large erosions > 2 mm.

Histopathologic analysis

Thereafter each stomach was divided in two equal portions. Three slides of tissue approximately 10×5 mm were cut out from ventral wall and were fixed in a 10% formalin solution and embedded in paraffin wax. Subsequently two sections at 4 μm were cut out from each coded slide and stained by haematoxylen-eosine. The severity of gastric mucosal injury was evaluated blindly by a pathologist using the criteria of Lacy and Ito (1982): Grade 0=normal mucosa, Grade 1=presence of desquamation on surface mucosal cells, but foveolar epithelium was intact, Grade 2=presence of desquamation on surface mucosal cells and foveolar epithelium, but gastric glands were intact and Grade 3=necrotic debris formation on surface epithelium or presence of gastric gland injury. For each rat the average of 6 examined sections formed microscopic data.

Biochemical assay

The dorsal wall of the stomach was stored in a deep freeze at -70°C until biochemical assay. Sialic acid and Malondialdehyde (MDA) were measured by the thiobarbituric acid methods described by Warren (1959), and Buege and Aust (1978), respectively. MDA results were expressed as nmol/g wet tissue. Sialic acid results were expressed as μg NANA/mg protein. SOD activity was calculated by using the nitroblue tetrazolium assay (Sun et al. 1988). The protein contents that used to deter-

mine the SOD activity and sialic acid were measured by the method of Lowry et al. (1951) using bovine albumin as a standard. SOD results were expressed as U/mg protein. Catalase activity was measured by the breakdown of hydrogen peroxide catalysed by catalase (Ronald et al. 1952). Catalase results were expressed as U/g wet tissue.

Statistical analysis

All data were expressed as median and as (25 th-75 th quartiles). To detect statistically significant differences among five groups the Kruskal Wallis H test was used. Differences with a *p*-value 0.05 were considered significant. Subsequent comparisons were made by using nonparametric Mann Whitney U test with adjusting the probability level downward and a probability level of *p*<0.005 was assumed to be significant (Dawson-Saunders and Trapp 1998). All *p*-values were two - tailed. In statistical analysis SPSS released 10.0 programme for Windows (SPSS Inc. Chicago, IL, USA) was used.

RESULTS

Results belonging to groups are shown in Table 1.

Gastric mucosal acidity.

Gastric mucosal acidity measurements are summarised in Figure 1. Four rats having pH values more than 5 had no macroscopic injury. None of the groups showed significant difference for gastric mucosal acidity (*p*>0.05).

Sialic acid.

There was no statistically significantly difference between groups according to sialic acid (*p*>0.05) (Fig. 2).

Gross damage.

Gross gastric lesions occurred on all over the fundus and corpus of the stomach. There was no statistically significantly difference between groups according to gross pathology (*p*>0.05) (Fig. 3).

TABLE 1. Results belonging to groups

	Group I	Group II	Group III	Group IV	Group V	
Gastric mucosal pH	3.00 (2.75-5.25)	3.00 (2.75-4.25)	3.00 (2.00-4.00)	3.50 (2.75-4.00)	3.00 (2.75-4.00)	$X^2=0.67$ df =4 $p =0.95$
Sialic Acid	18.26 (15.31-21.59)	18.77 (15.88-23.16)	17.57 (15.16-18.58)	19.33 (14.45-21.97)	21.15 (18.19-24.14)	$X^2=6.28$ df =4 $p =0.17$
Gross damage	5.00 (2.18-4.25)	5.00 (3.00-6.00)	3.50 (2.75-5.25)	4.00 (2.75-6.00)	4.50 (2.00-6.00)	$X^2=1.27$ df =4 $p =0.86$
Microscopic damage	2.00 (1.50-2.25)	2.00 (1.00-2.00)	2.00 (1.00-2.00)	4.00 (1.00-2.00)	2.00 (1.00-2.00)	$X^2=1.11$ df =4 $p =0.89$
MDA	2.33 (2.03-2.55)	2.63 (2.14-3.09)	3.07 (2.00-4.00)	3.50 (2.75-4.00)	3.00 (2.75-4.00)	$X^2=8.31$ df =4 $p =0.08$
SOD	3.29 (2.18-4.25)	5.57 (9.64-6.20)	7.51 (6.19-8.70)	4.44 (3.94-8.55)	7.58 (5.99-9.02)	$X^2=17.84$ df =4 $p =0.001$
Catalase	9.54 (7.72-10.68)	9.07 (7.69-10.17)	9.99 (8.49-11.19)	9.39 (8.09-11.58)	9.44 (6.49-11.28)	$X^2=1.35$ df =4 $p =0.85$

Data are expressed as median and (25-75) quartiles.

Kruskal Wallis H test is used in statistical analysis. X^2 , chi-square; df, degree of freedom.

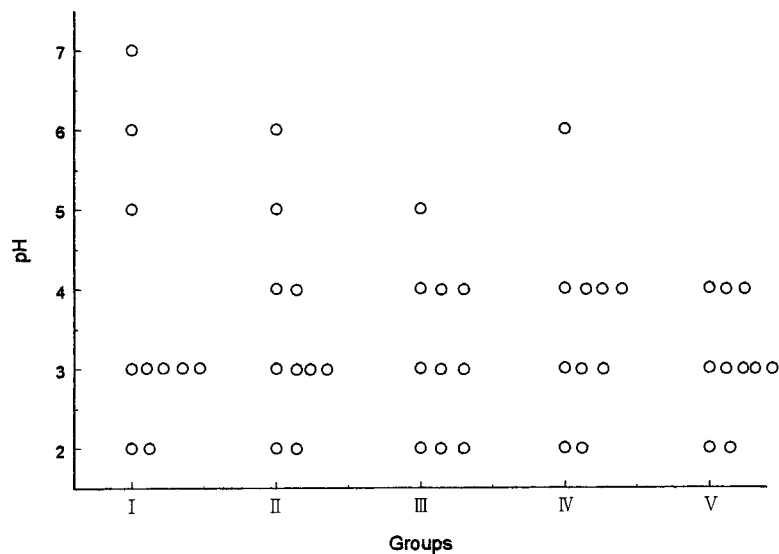


Fig. 1. Gastric mucosal acidity.

Histopathologic damage.

No statistically significant difference was found out between groups in view of histopathologic injury ($p > 0.05$) (Fig. 4).

MDA.

MDA results are summarised at Fig. 5. There was no statistically significant difference between groups ($p > 0.05$).

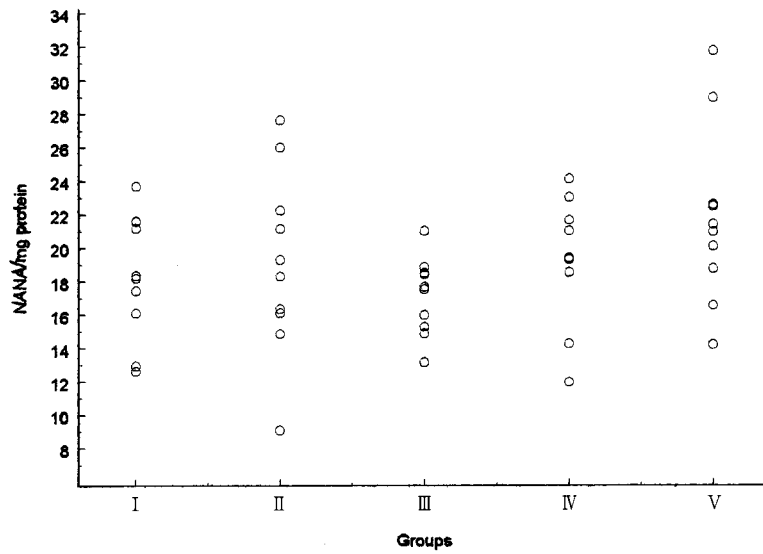


Fig. 2. Sialic acid results.

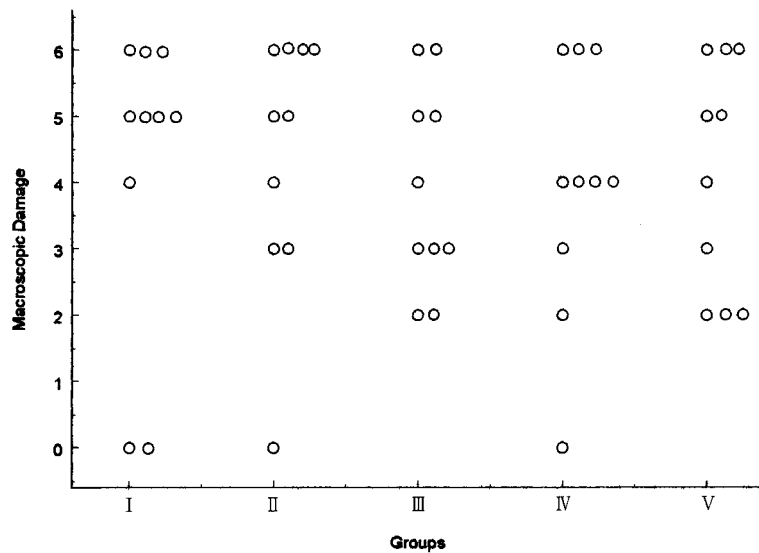


Fig. 3. Gross pathology.

SOD.

Gastric mucosal SOD results are summarised at Fig. 6. There was a significant difference between the groups ($p < 0.05$). Gastric mucosal SOD activity in group I was significantly lower compared to group III and Group V ($p < 0.005$).

Catalase.

Gastric mucosal catalase results are summarised at Fig. 7. There was no statistically significant

difference between groups ($p > 0.05$).

DISCUSSION

At the beginning of the 20th century, the human literature showed that gastrointestinal ulcer were more common in women but this difference has reduced in western population (Kurata and Haile 1985).

In the experimental stress literature the results of investigations are markedly different.

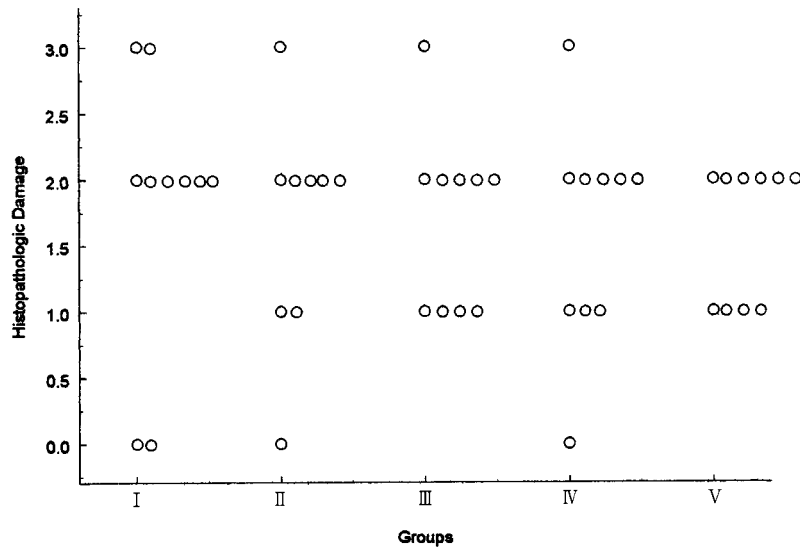


Fig. 4. Histopathologic damage.

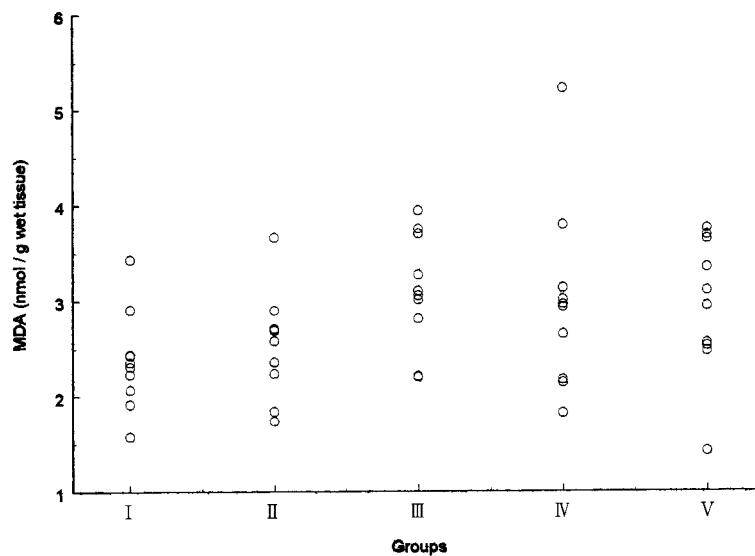


Fig. 5. MDA results.

One investigator has reported that male rats developed more stress ulcer when they were stressed by conflict stress model. Other investigators found out that female rats were more vulnerable to immobilisation ulcer and activity-stress ulcer (Lambert 1968; Herner and Caul 1972; Pare et al. 1978; Lambert and Kinsley 1993; Pare and Redei 1993). According to Pare and Redei (1993) the inconcordance in the results of experimental stress ulcer is related to

three factors. The first factor is the difference in the model used for stress ulcer formation. For this reason, the comparison of results is very difficult. The second factor is the usage of different strains of rats in these experiments. The third factor that the estrous cycle of the female rats was not taken into consideration in the majority of the above mentioned experiments.

In our study both gross and microscopic

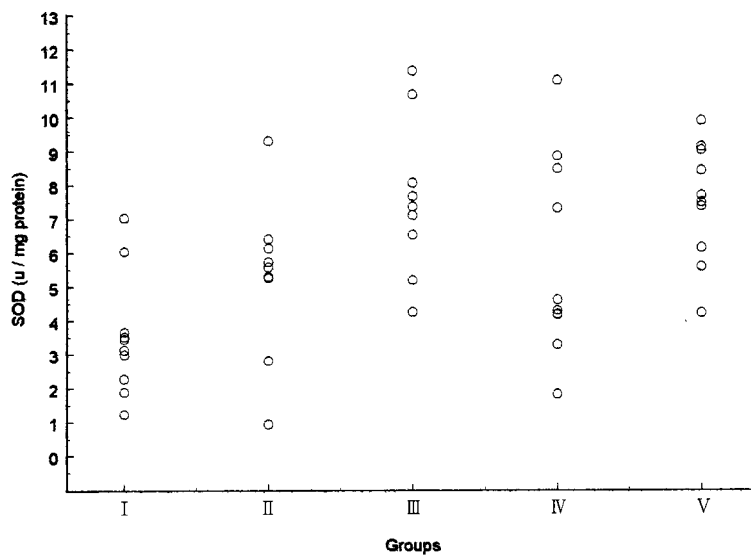


Fig. 6. SOD results.

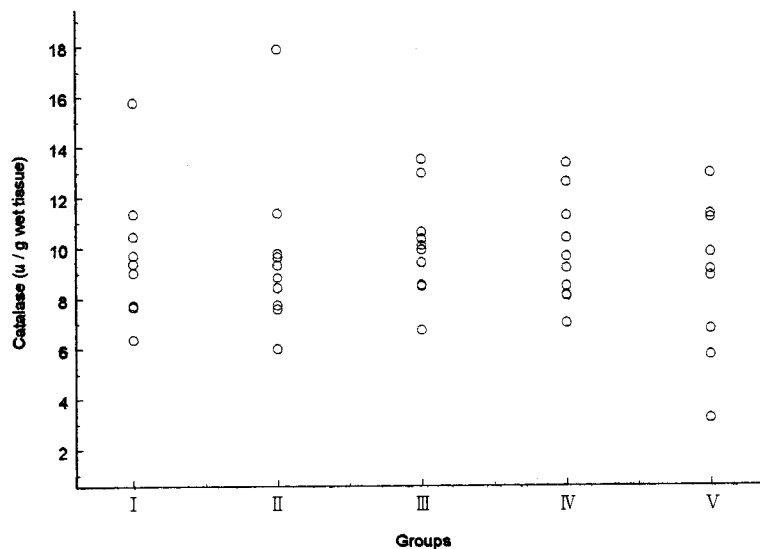


Fig. 7. Catalase results.

assessment of stress ulcer was done. No difference was significant among groups according to gross and microscopic damage ($p > 0.05$).

Restraint stress was found to increase gastric acid secretion (Lambert and Kinsley 1993), to reduce the gastric adherent mucus and to decrease the concentration of sialic acid (Somasundaram and Ganguly 1985), to stimulate oxygen derived free radicals production (Coskun et al. 1995). The presence of luminal

acid seems to be a prerequisite for the development of acute gastric mucosal lesions (Mersereau and Hincley 1973; Cheung 1988). Our study found out that when rats were stressed gastric mucosal pH values were not varied by gender ($p > 0.05$). We did not observe any ulceration in cases which luminal acid pH were above than five. Gastric mucosal surface cells are protected by gastric acid secreted in to the lumen of the stomach by their surface membrane and tight

intercellular junctions and the surface layer of mucus (Nord and Sodeman 1985). Removal of sialic acid content of gastric mucus from epithelial cell membrane was shown to lead to the loss of gastroprotection (Cho and Pfeiffer 1984; Ishidara and Ohara 1984). Sialic acid was measured in the gastric mucosa as an index of gastric mucosal protection. No statistically significant difference was found out between groups regarding NANA assesment ($p > 0.05$).

Many experimental studies reported that oxygen derived free radicals cause tissue injury through lipid peroxidation of plasma membrane (Itoh and Guth 1984; Salim 1990). MDA measurement reflects lipid peroxidation (Fridovich 1978). In our study there was no statistically significant difference among groups according to MDA levels ($p > 0.05$). Administration of radical scavengers was reported to protect gastric injury due to cold restraint models (Itoh and Guth 1984; Salim 1990; Ohara and Yuki 1991). SOD is a natural element of antioxidant system and a radical scavenger enzyme (Fridovich 1978). A study reported that administration of heparin which increase SOD activity in vivo reduce gastric mucosal lesions occurrence compared to control (Ohara and Yuki 1991). SOD level in group I was significantly lower compared to groups III and IV. Significantly higher level of SOD in group III compared to group I might be due to two factors. First was the effect of testosterone which decrease gastric SOD level in male rats (Group I). Our data was supported by a recent study which reported that administration of testosterone reduces SOD and catalase levels in rat testis (Chainy et al. 1997). Second was the effect of some female hormones which increase SOD levels in female groups (Group III and V). In estrus phase, female rats are exposed to higher levels of estrogen, progesterone and gonadotropin hormones whereas in diestrus phase, female rats are under exposure of reduced estrogen level (Sportnitz et al. 1999). Estrogen was showed to increase SOD activity and it has much more

effective antioxidant property than α -tocopherole (Ghanam et al. 1998). Thus specifically estrogen should be the responsible SOD increasing hormone in group III. Significantly increased SOD activity in group V compared to group I may be attributable to two factors. First was the effect of testosterone mentioned above, reduce SOD activity in male rats (Group I). Second was in the absence of both progesterone and estrogen following castration procedure, female gonadotropin hormones must be the SOD increasing hormone in group V, because an elevation of circulating gonadotropins was a characteristic of post castration period (Gay and Midgley 1969; Yamamoto et al. 1970; Pajovic et al. 1993). However in groups III and V, gross and microscopic gastric damage were not significantly different compared to group I. Aguwa (1984) reported that exogenous administration of female sex hormones (progesterone and estrogen) were found to have significant antiulcer activity in almost all different ulcer models. Aguwa's study (1984) had thought us that the physiologic quantity of female hormones in groups III and V were insufficient to protect effectively against gastric ulcer formation.

Catalase is also a member of natural antioxidant system (Fridovich 1978). There was no statistically significant difference between groups according to catalase ($p > 0.05$).

We concluded that stress ulcer formation was not influenced by gender nor by estrus cycle of female rats. SOD levels were significantly higher in group III and in group V compared to group I. This results indicated that estrogen and female gonadotropin hormones increase SOD and testosterone reduce it. In higher levels of SOD groups, the physiologic quantity of mentioned above female hormones may be not sufficient to protect effectively against stress ulcer production.

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References

- Aguwa, C.N. (1984) Effects of exogenous administration of female sex hormones on gastric secretion and ulcer formation in the rat. *Eur. J. Pharmacol.*, **104**, 79-84.
- Andrews, F.J., Malcontenti, C. & O'Brien, P.E. (1992) Sequence of gastric mucosal injury following ischemia and reperfusion: Role of reactive oxygen metabolites. *Dig. Dis. Sci.*, **37**, 1356-1361.
- Brown, K. & Fridovich, I. (1980) Superoxide radical and superoxide dismutase. Threat and defense. *Acta. Physiol. Scand.*, **492**, 9-18.
- Brodie, D.A. & Hanson, H.M. (1960) A study of the factors involved in the production of gastric ulcers by the restraint technique. *Gastroenterology*, **38**, 353-360.
- Buege, J.A. & Aust, S.D. (1978) Microsomal lipid peroxidation. *Methods. Enzymol.*, **52**, 302-310.
- Chainy, G.B., Samantaray, S. & Samanta, L. (1997) Testosterone induced changes in testicular antioxidant system. *Andrologia*, **29**, 343-349.
- Cheung, L.Y. (1988) Pathogenesis, prophylaxis and treatment of stress gastritis. *Am. J. Surg.*, **156**, 437-440.
- Cho, C.H. & Pfeiffer, C.I. (1984) Study of the damaging effects of acetotolamide on gastric mucosa in rats. *Acta Physiol. Hung.*, **64**, 279-285.
- Coskun, T., Alican, I., Yegen, B.C., San, T., Cetinel, S. & Kurtel, H. (1995) Cyclosporin A reduces the severity of cold restraint induced gastric lesions: role of leukocytes. *Digestion*, **56**, 214-219.
- Dawson-Saunders, B. & Trapp, R. (1998) In: *Basic and Clinical BioStatistic*. Appleton Lange, Norwalk, Connecticut, pp. 133-139.
- Fenton, A.H., Clark, T.W., Meyer, M.S. & Woorman, D.W. (1992) Prevention of stress gastritis with tissue preservation solution. *Am. Surg.*, **58**, 250-254.
- Dekansky, J.B., Mac Donald, D. & Sacra, P. (1975) Effects of fasting, stress and drugs on gastric glycoprotein synthesis in the rat. *Br. J. Pharmacol.*, **55**, 387-392.
- Fridovich, I. (1978) The biology of oxygen radical. *Science*, **201**, 875-880.
- Gay, V.L. & Midgley, A.R., Jr. (1969) Response of the adult rat to orchidectomy and ovariectomy as determined by LH radioimmunoassay. *Endocrinology*, **84**, 1359-1364.
- Ghanam, K., Javellud, J., Ea-Kim, L. & Oudart, N. (1998) The protective effect of 17 beta-estradiol on vasomotor responses of aorta from cholesterol-fed rabbit is reduced by inhibitors of superoxide dismutase and catalase. *Biochem. Biophys. Res. Commun.*, **249**, 858-864.
- Herner, D. & Caul, W.F. (1972) Restraint induced ulcer in rats during estrus and diestrus. *Physiol. Behav.*, **8**, 777-779.
- Ishidara, K., Ohara, S., Azuumi, Y., Gosok, K. & Hotta, K. (1984) Changes of gastric mucus glycoproteins with aspirin administration in rats. *Digestion*, **29**, 98-102.
- Itoh, M. & Guth, P.H. (1984) Superoxide radicals play a role in hemorrhagic shock induced gastric lesions in the rat. *Gastroenterology*, **86**, 1122.
- Kurata, J.H., Haile, B.M. & Elashoff, J.D. (1985) Sex differences in peptic ulcer disease. *Gastroenterology*, **88**, 96-100.
- Lacy, E.R. & Ito, S. (1982) Microscopic analysis of ethanol damage to rat gastric mucosa after treatment with a prostaglandin. *Gastroenterology*, **83**, 619-625.
- Lambert, R. (1968) Use of the rat in the exploration of experimental peptic ulcer and sequelae of gastrectomy. In: *Progress in Gastroenterology*, vol. 1., edited by G.B.J. Glass. New York, Grune and Stratton, pp. 40-66.
- Lambert, K.G. & Kinsley, C.H. (1993) Sex differences and gonadal hormones influences susceptibility to activity-stress paradigm. *Physiol. Behav.*, **53**, 1085-1090.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with folin phenol reagent. *J. Biol. Chem.*, (193) 265-275.
- Mersereau, W.A. & Hinchey, E.J. (1973) Effect of gastric acidity on gastric ulceration induced by hemorrhage in the rat utilizing a gastric chamber technique. *Gastroenterology*, **64**, 1130-1135.
- Nord, T.H. & Sodeman, W.A. (1985) The stomach. In: edited by W.A. Sodeman and T.M. Sodeman, *Sodeman's Pathologic Physiology of Disease*. 7th ed., WB Saunders, Igaku Shoin, pp. 787-812.
- Ohara, T., Yuki, T., Hamada, S., Asaki, S. & Toyota, S.A. (1991) Cytoprotective role of extracellular superoxide dismutase in experimental stress ulcer models. *Nippon Shokakibyo*

- Gakkai Zasshi*, **88**, 659-663. (Abstract)
- Pajovic, S., Nikezic, G. & Martinovic, J.V. (1993) Effects of ovarian steroids on superoxide dismutase activity in the rat brain. *Experientia*, **49**, 73-75.
- Pare, W.P., Wincent, G.P., Isom, K.E. & Reeves, J.M. (1978) Sex differences and incidence of activity-stress ulcers in rats. *Psychol. Rep.*, **43**, 591-594.
- Pare, W.P. & Redei, E. (1993) Sex differences and stress response of WKY rats. *Physiol. Behav.*, **54**, 1179-1185.
- Perry, M.A., Wadhwa, S., Parks, D.A., Pickard, W. & Granger, N.D. (1986) Role of oxygen radicals in ischemia induced lesions in the cat stomach. *Gastroenterology*, **90**, 362-367.
- Poole, T.B. (1999) In: *UFAW Handbook on the Care and Management of Laboratory Animals*, 7th ed., Blackwell Science, UK.
- Roland, F., Beers, R.F., Irwin, W. & Sizer, A. (1952) A Spectrophotometric method measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.*, **195**, 133-140.
- Salim, A.S. (1990) Removing oxygen derived free radicals stimulates healing of ethanol-induced erosive gastritis in the rat. *Digestion*, **47**, 24-28.
- Sawrey, W.L. & Long, D.H. (1962) Strain and sex differences in ulceration in the rat. *J. Comp. Physiol. Psychol.*, **55**, 603-605.
- Somasundaram, K. & Ganguly, A.K. (1985) Gastric mucosal protection during restraint stress in rats: alteration in gastric-adherent mucos and dissolved mucus ingastric secretion. *Hepatogastroenterology*, **32**, 24-26.
- Sportnitz, U.M., Socin, C.D. & Dravid, A.A. (1999) Estrous stage determination in rats by means of scanning electron microscopic images of uterine surface epithelium. *Anat. Record*, **254**, 116-126.
- Sun, Y., Oberley, L.W. & Li, Y. (1988) A simple method for clinical assay of superoxide dismutase. *Clin. Chem.*, **34**, 497-500.
- Warren, L. (1959) The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.*, **235**, 1971-1975.
- Yamamoto, M., Diebel, N.D. & Bogdanove, E.M. (1970) Analysis of initial and delayed effects of orchidectomy and ovariectomy on pituitary and serum LH levels in adult and immature rats. *Endocrinology*, **86**, 1102-1111.
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