

## Manda Suppresses Emotional Stress-Induced Stomach Ulcers in Rats

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*Manda, a natural product made by yeast fermentation of many fruits and black sugar, has antioxidant activity. In the present study, manda prevented stomach ulcers caused by immobilization-induced emotional stress. Manda [5% manda solution (w/v)] and saline as control, were administered by a canula into the stomach of each experimental animal subsequently after 1, 2, 3, 4, and 5 hours from the start of the emotional stress. We classified the severity of gastric lesion formation induced by immobilization with each rat lying on its back for 6 hours at room temperature on a five-grade scale. The control rats all showed congestion and some degree of bleeding in the mucosa of the stomach. However, of the experimental rats, one showed no hemorrhagic lesions only congestion in four cases, and slight or moderate bleeding in eight cases with no massive bleeding cases. The distribution of these data significantly differ from that of the control rats, which suffered the greater damage ( $\chi^2 = 10.589$ ,  $p < 0.05$ ). In light microscopic examinations, the control rats showed necrosis in the gastric mucous membranes, desquamation, and bleeding of gastric mucosa. However, the rats treated with manda showed only congestion and did not show erosion or hemorrhage. These results suggest that manda or manda metabolite(s) was absorbed from the stomach and may have produced these action. In the meantime, we are analyzing manda components to try to isolate the active ingredient(s)*

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**KEY WORDS:** stress; ulcers; antioxidant; free radical.

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## INTRODUCTION

Manda is a natural product made by yeast fermentation of many fruits and black sugar. The starting materials, its composition and the method of preparation have previously been reported (Kawai, Matsuura, & Mori, 1994). Manda was demonstrated to scavenge hydroxyl, superoxide, and diphenyl-*p*-picrylhydrazyl (DPPH) radicals *in vitro*, and to inhibit the formation of thiobarbituric acid-reactive substances (TBARS) in iron-induced epileptic foci in rats (Kawai, & Matsuura, 1997). Furthermore, its beneficial effects on plants and experiment animals, including antioxidant effect on reactive oxygen species (ROS) have been reported (Kawai, & Matsuura, 1997; Kawai, Matsuura, & Mori, 1994).

Recently, the pathogenesis of stomach ulcer has been related to ROS generation produced from blood flow, ischemic tissue or leukocytes such as neutrophils. Furthermore, it has been shown that the gastric mucosa was injured by free radicals, and that such an injury was inhibited by antioxidant reagents (Granger, Rutiki, & McCord, 1981; Henrotte, Franck, Santarromana, Nakib, Dauchy, & Boulu, 1992; Liu, & Mori, 1994; Liu, Wang, & Mori, 1994; Obispo, 1993; Ray, & Henke, 1990; Salim, 1989; Yoshikawa, 1989; Yoshikawa, Miyagawa, Yoshida, Sugino, & Kondo, 1986; Yoshikawa, Yoshida, Miyagawa, Takemura, Sugino, & Kondo, 1987). In this paper, we investigated the prophylactic effect of Manda, an antioxidant, on the stomach ulcer induced by immobilization stress, which is known to relate closely to free radicals. Furthermore, we investigated the stomach cell lesion induced by stress and confirmed the protective action of manda by light microscopy.

## METHODS

*Animals.* Twenty-four male Sprague Dawley rats age of 7–10 weeks, weighing 210–300 g were used, and were housed at constant temperature ( $25 \pm 2^\circ\text{C}$ ) and humidity ( $50 \pm 5\%$ ) prior to this experiment for 1 week. Eleven were randomly assigned to the experimental group and 13 to the control group.

*Procedure.* All rats were slightly anesthetized with ethylether. Emotional stress was charged by immobilization with each rat lying on its back for 5 hours at room temperature ( $20^\circ\text{C} \pm 2^\circ\text{C}$ ) according to Weininger's method (Weininger, 1956). Manda (5% manda solution (w/v), Manda Fermentation Co., Ltd., Hiroshima) was administered by a canula into the stomach of each experimental animal after 1, 2, 3, 4, and 5 hours from the starting of the emotional stress. The control rats were administered with saline instead of manda on the same schedule.

*Preparation of Tissue Sections.* After 6 hours, all experimental rats were

slightly anesthetized with ethylether, and promptly sacrificed. Their stomachs were removed, dissected along their greater curvature, the inner mucous membranes were opened and prepared for sampling of microscopical observation, i.e., the stomachs were dissected transversely into several blocks and fixed by a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer solution. After fixation, these blocks were removed and washed in 0.1 M phosphate buffer solution. The blocks were then dehydrated in ethanol and embedded in paraffin. Five-micrometer-thick sections were prepared by a microtome and mounted on glass slides coated with egg albumin. The slides were fixed by xylen and stained with hematoxylin and eosin, then coverslipped by Entellan and observed by light microscopy.

## RESULTS

*Analysis of stomach ulceration.* The severity of stomach ulcers was classified on a five-grade scale ranging from no pathology (0) to massive bleeding (5), as in Table I.

The control rats who did not receive manda all showed congestion and some degree of bleeding in the mucous membrane of stomach (Table I). On the other hand, the mucosa of the 13 experimental rats one showed no hemorrhagic lesions, only congestion (four cases), and slight or moderate bleeding (eight cases) without massive bleeding cases. The distribution of the data for the experimental cases differs significantly from that of the control rats, which suffered the greater damage ( $\chi^2 = 10.589$ ,  $p < 0.05$ ).

Figures 1 and 2 show two examples from the light microscopic examinations. The control rats showed necrosis in the gastric mucous membranes, desquamation, and bleeding of the gastric mucosa. However, the rats treated with manda showed only congestion and did not show erosion or hemorrhage.

Table I. Pathological Findings in Inner Mucosa of Rat Stomachs Induced by 5 Hours Immobilization Stress\*

Condition	Control group (N=11)	Experimental group (N=13)
No pathology	0	1
Congestion	1	4
Slight bleeding	3	6
Moderate bleeding	2	2
Massive bleeding	5	0

\* $\chi^2 = 10.589$ ;  $p < 0.05$ .

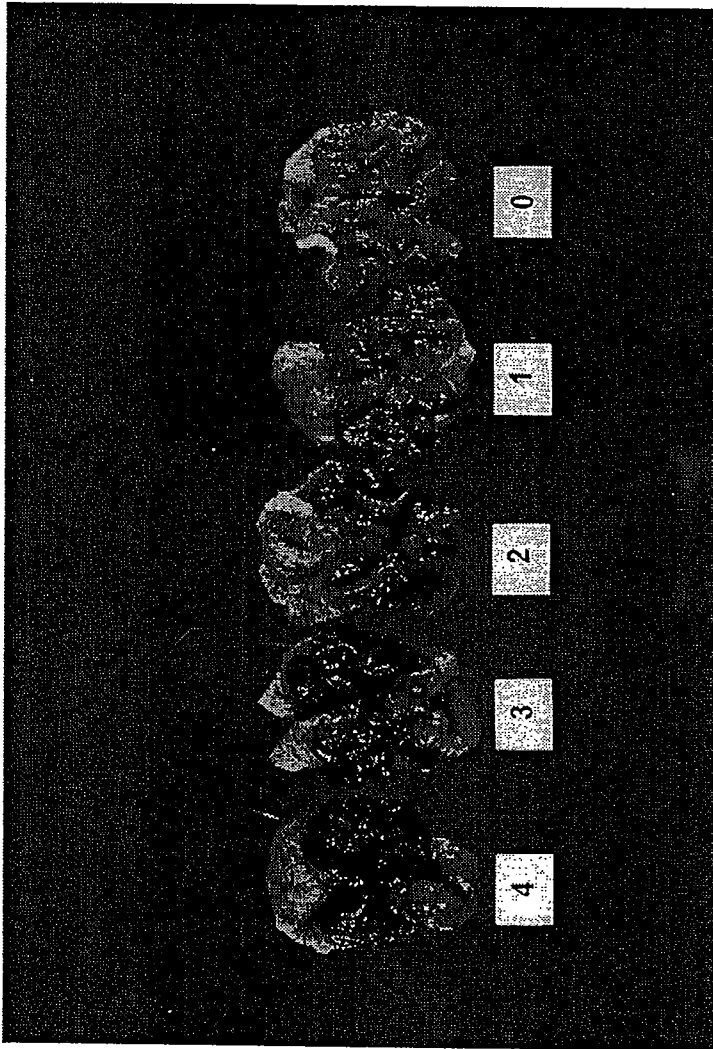


Fig. 1. Severity points of ulcer in the inner mucosa of rat stomach after immobilization for 6 hours.



Fig. 2. Examples of light micrographs of gastric mucous membranes. (A) Gastric mucous membranes of stressed rats (control). Note the desquamation and necrosis of mucous membrane in this control rat. (B) Gastric mucous membranes of stressed rats administered 5% manda solution. No necrotic lesions were observed in the mucosa of this experimental rat. Also, erosion was observed in the control rat (A), but only congestion or slight bleeding were observed in manda-administered group (B).

## DISCUSSION

In the present study, we observed that manda, an antioxidant (Kawai, Matsuura, & Mori, 1994; Kawai, & Matsuura, 1997), prevented stomach ulcers by immobilization-induced emotional stress.

It has been reported that ROS induce acute stomach mucous membrane injury, i.e., the ischemic or low blood flow increases hypoxanthine/xanthine oxidase activity and ROS generate explosively by reperfusion. Furthermore, disturbed circulation and energy metabolism in the gastric mucosa are considered a cause of acute mucosal lesion during hemorrhagic shock (Sato & Kamada, 1979). Moreover, ROS may generate microvascular endothelial damages, without an abrupt oxygen supply by reperfusion, because of rearranged oxygen supply in the microvascular system (Kurose, & Suematsu, 1991).

Two ways for participation of ROS in tissue damage induced by ischemia or low blood flow have been proposed. One is the leakage of ROS in the process with acceleration of platelet activating factor (PAF) systems and activation of tissue plasminogen activator (t-PA). Localized activation of collagenase by plasmin also may be involved in this procedure (Lewis & Whatley, 1988). These processes are not dependent on any events in neutrophil. The other one is direct cytotoxicity of activated neutrophils on the affected part. Neutrophils are known to be activated by adhesion molecules and to generate ROS, such as superoxide ( $O_2^-$ ), hydroxyl radical ( $\cdot OH$ ), or hypochlorous anion ( $ClO^-$ ). In this procedure, exogenous antioxidants were effective for modulating neutrophil activation *in vivo*, even if they were not direct radical scavengers (Kurose, Suematsu, Miura, Fukumura, Sekizuka, Nagata, Oshio, & Tsuchiya, 1993, Suematsu, & Kurose, 1989). ROS-induced disorders are prevented by the administration of xanthine oxidase inhibitors or hydroxyl radical scavengers (Salim, 1989). In the present study, it was demonstrated that manda inhibited emotional stress-induced stomach ulcers in rats.

In conclusion, manda, probably by a radical scavenging mechanism, prevented the development of emotional stress-induced stomach ulcers, a finding thoroughly confirmed by the histological findings.

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## REFERENCES

- Granger, D. N., Rutiki, G., & McCord, J. M. (1981). Superoxide radicals in ferine intestinal ischemia. *Gastroenterology*, *81*, 22–29.
- Henrotte, J. G., Franck, G., Santarromana, M., Nakib, S., Dauchy, F., & Boulu, R. G. (1992). Effect of pyridoxine on mice gastric ulcer and brain catecholamines after an immobilization stress. *Annals of Nutrition and Metabolism*, *36*, 313–317.
- Kawai, M., Matsuura, S., & Mori, A. (1994). Free radical scavenging action of Manda. *The Clinical Report*, *28*, 393–397.
- Kawai, M., & Matsuura, S. (1997). Manda scavenges free radicals and inhibits lipid peroxidation in iron-induced epileptic focus in rats. In M. Hiramatsu, T. Yoshikawa, & M. Inoue, (Eds.), *Foods and free radical* (pp. 141–145). New York: Plenum Press.
- Kurose, I., & Suematsu, M. (1991). Involvement of superoxide anion and platelet-activating factor increased tissue-type plasminogen activator during rat gastric microvascular damages. *Thrombosis Research*, *62*, 241–248.
- Kurose, I., Suematsu, M., Miura, S., Fukumura, D., Sekizuka, E., Nagata, H., Oshio, C., & Tsuchiya, M. (1993). Oxyradical generation from leukocytes during endotoxin-induced microcirculatory disturbance in rat mesentery—Attenuating effect of cetraxate. *Toxicology and Applied Pharmacology*, *120*, 37–44.
- Lewis, M. S., & Whitley, R. E. (1988). Hydrogen peroxide stimulates the synthesis of platelet activating factor by endothelium and induces endothelial cell-dependent neutrophil adhesion. *Journal of Clinical Investigation*, *82*, 2045–2055.
- Liu, J., & Mori, A. (1994). Involvement of reactive oxygen species in emotional stress: A hypothesis based on the immobilization stress-induced oxidative damage and antioxidant defense changes in rat brain, and the effect of antioxidant treatment with reduced glutathione. *International Journal of Management*, *1*, 249–263.
- Liu, J., Wang, X., & Mori, A. (1994). Immobilization stress induced antioxidant defense changes in rat plasma: effect of treatment with reduced glutathione. *International Journal of Biochemistry*, *26*, 511–517.
- Obispo, M. J. M. (1993). Gastric ulcers caused by post-burning stress: contribution of free radical. *Revista Espanola de Enfermedades Digestivas*, *84*, 287–289.
- Ray, A., & Henke, P. G. (1990). Enkephalin-dopamine interactions in the central amygdalar nucleus during gastric stress ulcer formation in rats. *Behavioural Brain Research*, *36*, 179–183.
- Salim, A. S. (1989). Scavenging free radicals to stress-induced gastric mucosal injury. *Lancet*, *2*, 1390.
- Sato, N., & Kameda, T. (1979). Measurement of hemoperfusion and oxygen sufficiency in gastric mucosa in vivo. *Gastroenterology*, *76*, 814–819.
- Suematsu, M., & Kurose, I. (1989). In vivo visualization of oxyradical-dependent photoemission during endothelium-granulocyte interaction in microvascular beds treated with platelet-activating factor. *Journal of Biochemistry*, *106*, 355–360.
- Weininger, O. (1956). The effects of experiment of behavior and growth characteristics. *Journal of Comparative Physiology & Psychology*, *49*, 1–9.
- Yoshikawa, T., Miyagawa, H., Yoshida, N., Sugino, S., & Kondo, M. (1986). Increase in lipid peroxidation in rat gastric mucosal lesions induced by water-immersion restraint stress. *Journal of Clinical Biochemistry and Nutrition*, *1*, 271–277.
- Yoshikawa, T., Yoshida, N., Miyagawa, H., Takemura, T., Sugino, S., & Kondo, M. (1987). Role of lipid peroxidation in gastric mucosal lesions induced by burn shock in rats. *Journal of Clinical Biochemistry and Nutrition*, *2*, 163–170.
- Yoshikawa, T., Ueda, S., Naito, Y., Takahashi, S., Oyamada, H., Morita, Y., Yoneta, T., & Kondo, M. (1989). Role of oxygen-derived free radicals in gastric mucosal injury induced by ischemia or ischemia-reperfusion in rats. *Free Radical Research Communications*, *7*, 285–291.