J. Korean Soc. Food Nutr.
22 (6), 823 ~ 832 (1993)

Alleviating Effects of Vitamin C on the Gramoxone Toxicity in the Total Lipid Contents, Lipid Peroxidation and Protein Patterns of Rat Liver

Un-Bock Jo†, Sung-Ro Kim* and Byung-Tae Choi

Dept. of Biology Education, Pusan National University, Pusan 609-735, Korea
**Dept. of Food and Nutrition, Tongnae Women's Junior College, Pusan 612-084, Korea*

Abstract

This study was made to determine alleviating effects of vitamin C (Vt C) on the gramoxone toxicity in rats with respect to the growth gain, feed efficiency ratio, total lipid contents, lipid peroxidation and protein patterns in rat liver. Growth gain, feed efficiency ratio and liver weight in the gramoxone group were decreased significantly as compared with the control group, while those values obtained in the gramoxone-Vt C group were increased as compared with the gramoxone group. Lipid contents and TBA value of rat liver of the gramoxone group were increased significantly as compared with the control group. However, those values of the gramoxone + Vt C group were decreased as compared with the gramoxone group. No significant differences were observed in the liver protein patterns between the gramoxone and the control group. The contents of liver protein were decreased significantly in the gramoxone group fed for 2 weeks as compared with the control group. The changes of the liver protein patterns, such as the decrease of high molecular weight protein and the increase of low molecular weight protein were observed in the gramoxone + Vt C group.

Key words : gramoxone, vitamin C, rat liver

INTRODUCTION

Most pesticides have important effects on the environmental contamination. These pesticides bring about toxic effects in living organisms and generally the causes of the several diseases and death[1~3]. Among these pesticides, gramoxone (paraquat) is the most commonly used herbicide for destruction of noxious weeds. Murphy[3] reported that gramoxone caused necrotic effects in animal lung, liver and kidney. It has been also reported that the manifestations of gramoxone toxicity were related to the suppressed activities of alkaline phosphatase and acid phosphatase, and involved the changes of mucosubstances in rat intestines[4~6]. Kim[7] reported that gramoxone had necrotic effects on lipid contents and glycogen metabolism of liver, and activities of sGOT, sGPT, cholinesterase, alkaline phosphatase and acid phosphatase. It has been also proposed that the suggested mechanism of gramoxone toxicity is related to the formation of superoxide, hydrogen peroxides and NADPH dependent lipid peroxides, and the damage to hemoglobin by free radical, the catabolism of protein and hemolysis by lipid peroxides in cell membrane[8,9].

Recently, there has been heightened risk of exposure to foreign compounds. Among the several factors which affect the manifestations of foreign compounds, the nutritional status of a living organism is deemed to be one of the important factors in representing the toxicity of foreign compounds[10,11]. Marasmic diet, kwashiorkoric diet and vitamin deficiency are found to increase the toxicity of foreign compounds[9~13].

Vitamin C (L-ascorbic acid) is well known to antiscurvic agent, and plays an important role in numerous biological mechanisms including collagen synthesis, the healing of wounds, the union of fractures, the regeneration of nerves, the metabolism of folic acid, the absorption of iron, carnitine synthesis, norepinephrine synthesis and degradation of cholesterol[14~17]. It also takes part in the metabolism

† To whom all correspondence should be addressed

of tyrosine and acts as an antioxidant. Vitamin C converts active oxygen such as superoxide radical into less toxic or non-toxic to the cell[17]. Machlin[16] also reported that rats given daily doses of 6.5g of vitamin C/kg body weight were not showed any abnormal toxicities. He also described that vitamin C increased the mobility of white blood cells, serum levels of immunoglobulins and antibody formation[16].

Because the manifestations of chemical toxicity are significantly related to the nutritional status of individuals, this study is designed to determine the effect of dietary supplementation of vitamin C on the manifestation of gramoxone toxicity in rat liver. In order to show whether the toxicity of gramoxone can be modified by supplementations of vitamin C or not, weight gains, feed efficiency ratio, and lipid contents, TBA value and protein patterns of liver were examined.

MATERIALS AND METHODS

Feeding experiment

Thirty two Wistar-strained male rats, aged 6 to 7 weeks, were divided into control group(12) and experimental group(20). Experimental group was divided into the gramoxone-treated group(toxic group) and the gramoxone +Vt C-treated group (alleviating group). Rats in control group and gramoxone-treated group were fed 18% casein diet and 18% casein +0.04% gramoxone diet for 4 weeks, respectively. In case of the gramoxone +Vt C-treated group, rats were fed 18% casein +0.04% gramoxone +Vt C diet for 2 weeks after feeding 18% casein +gramoxone diet for 2 weeks. Experimental diets were prepared according to the composition shown in Table 1. Food intakes and body weight changes were recorded every other day during experimental days. At the end of desired experimental days, rats fasted for 18 hours and were anesthetized with ether. Rat liver was excised immediately after anesthetizing.

Determination of TBA value and crude lipid of the rat liver

The rat liver was homogenized in a glass homogenizer using extracting solution(chloroform/methanol=2/1, v/v). Total lipid was extracted with an extracting solution for one and half hours using a Soxtec system HT 2-1045 extraction unit 1 and a Soxtec system 1044 service unit 1. Hepatic TBA value was determined at 532nm. Every sample was duplicated for determining lipid contents in liver and triplicated for the hepatic TBA value.

SDS-polyacrylamide gel electrophoresis(PAGE) of protein patterns in the rat liver

SDS-PAGE of protein patterns was performed according to Laemmli method[18]. Two grams of liver tissue of each group were homogenized in a glass homogenizer using 4ml grinding buffer solution (50mM Tris-HCl, 0.1mM NaCl, 0.1mM EDTA, 2% SDS, 5% mercaptoethanol, pH 7.8). This homogenate was centrifuged at 10,000×g for 1hr at 4°C. The supernatant was further centrifuged at 10,000 ×g for 30min at 4°C. The supernatant was then mixed with an equal volume of twice-diluted stock solution of stacking gel buffer containing 20% sucrose. The protein samples were then boiled for 90 sec at 100°C, and cooled at room temperature. The sample was applied with 0.002% BPB(bromphenolblue) as a tracking marker before electrophoresis. The resolving gel consisted of 13% acrylamide and adjusted to pH 8.8. Four percent acrylamide was

Table 1. Composition of the experimental diets

Constituents (%)	CG	GG	GVG
Corn starch	72	72	69
Casein	18	18	18
Corn oil	5	5	5
Salt mixture[1]	4	4	4
Vitamin mixture[2]	1	1	1
L-ascorbic acid	–	–	3
Gramoxone		0.04	0.04

Abbreviations : CG, 18% casein diet(control group) ; GG, 18% casein+0.04% gramoxone diet(gramoxone group) ; GVG, 18% casein+0.04% gramoxone+3% L-ascorbic acid diet(gramoxone-vitamin C group)

[1] Salt mixture : Purchased from Nutritional Biochemicals Corp. Cleveland, Ohio, U.S.A.

[2] Vitamin mixture : Vitamin diet fortification mixture ; purchased from Nutritional Biochemicals Corp. Cleveland, Ohio, U.S.A.

prepared as a stacking gel and adjusted to pH 6.8. Thirty microliters of the sample were applied to the well. The stacking gel was then run at 70 volts for 2hrs and the resolving gel was run at 100 volts for 5 hrs After electrophoresis, the gel was stained with 0.1% coomassie brilliant blue R-250 for 3hrs and destained using a destaining solution which had 30% methanol and 10% acetic acid. The destained gel was scanned using a densitometer (Toyo DMU 33C, Japan) at 565nm. The molecular weight of the protein bands was estimated according to the method of Weber and Osborn[19]. Standard proteins such as bovine serum albumin (68,000 d), ovalbumin (43,000 d), carbonic anhydrase (29,000 d), lysozyme (14,300 d), and ribonuclease (13,700 d) were run simultaneously with the sample of the gel. The molecular weight of the sample was calculated from a plot of electrophoretic mobility versus molecular weight of the standard proteins.

Statistical analysis

To detect significant differences in the results of the this experiment, data were treated statistically using SPSS[20]. The subprogram, ONEWAY, which was specifically designed for a one-way analysis of variance, was employed using a significance level of 0.05. If significant differences were present, the LSD procedure was used at a significance level of 0.05 in order to determine the pattern of difference.

RESULTS AND DISCUSSION

Total weight gain and feed efficiency ratio of rats

Weight gain and feed efficiency ratio of rats fed control diet, gramoxone diet, and gramoxone +vitamin C diet for 4weeks are shown in Table 2. The highest weight gain (122.00 ± 4.55g) and the highest feed efficiency ratio (33.72 ± 2.84) was obtained in rats fed the control diet for 4 weeks and for 2weeks, respectively. On the other hand, the lowest weight gain (40.25 ± 8.73g) and the lowest feed efficiency ratio (15.05 ± 2.03) was obtained in rats fed the gramoxone diet for 2weeks and 4weeks, respectively. Results of the one-way analysis of variance indicated that significant differences($p<0.05$) were found in the total weight gain and the feed efficiency ratio of rats fed the control diet and the gramoxone diet. No significant differences, however, existed in the total weight gain and the feed efficiency ratio of rats fed the control diet and the gramoxone +Vt C diet.

The results were consistent with those found by other researchers who reported that the administration of specific foreign compounds resulted in a decrease in the body weight of the experimental animals. Cho *et al.*[21] reported that the total body weight of rats received mercuric chloride solution resulted in the reduction of the body weight. They also reported that the body weight of rats was decreased in proportion to the increment of mercury concentration. The addition of 0.1% PCB into the diet was shown to have caused the sebsequent decrease in the body weight of rats as compared with rats fed the control diet[9,13,22]. Additionally, Kim *et al.*[23] demonstrated that the total weight gain and the feed efficiency ratio of rats fed cadmium-added diet were decreased significantly as compared with the control group. The addition of gramoxone into the rats diets was shown to affect significant reduction in the body weight and the feed efficiency ratio. The supplementation of vitamin C into the rats diets containing gramoxone represented to have great effects on the improvement of body weight and feed efficiency ratio of rats (Table 2).

According to Fox *et al.*[24], vitamin C had marked effects in preventing growth retardation of young coturnix fed an adequate purified diet containing 75mg of cadmium/kg. Suzuki and Yoshida[25] reported that the supplementation of 400ppm of iron and 1% of vitamin C into the diet containing lead prevented the growth depression and anemia of rats. Moreover, Chatterjee *et al.*[26] presented that the supplementation of 20mg vitamin C/100g of body weight was shown to have an alleviating effect on the growth retardation in chronic chlordane toxified rats.

Thus, the supplementation of vitamin C into gramoxone diets proved to improve the body weight and feed efficiency ratio of rats, and this tendency appeared to be more evident in rats supplemented

vitamin C for 2 weeks rather than 1 week.

Liver weight and ratio of liver weight per body weight

The liver weight and ratio of liver weight per body weight of rats fed experimental diets are shown in Table 3. No significant differences existed in the liver of rats fed the control diet. Liver weight was, however, decreased significantly in rats fed the gramoxone diet. In case of the gramoxone + Vt C group, liver weight was decreased as compared with the control group, while it was increased as compared with the gramoxone group. The highest liver weight (8.45±0.63g) and the lowest liver weight (6.06±1.40g) was obtained in rats fed the control diet for 2weeks and the gramoxone diet for 3weeks, respectively. No significant differences existed in the ratio of liver weight per body weight of rats fed the control diet, the gramoxone diet, and the gramoxone + Vt C diet. With regard to the administration of foreign compounds, the liver weight of experimental animals tended to decrease, while the ratio of liver weight per body weight was increased[13,27,28]. Ando[13] reported that the liver weight of rats was decreased significantly when DDT was injected intraperitoneally. Kim[7] presented that the liver weight was decreased with the addition of gramoxone. The ratio of liver weight per body weight was increased with the addition of DDT[13], hexachlorocyclohexane[27], and BHT[28]. Kim[7], however, demonstrated that the ratio of liver weight per body weight was decreased with the addition of gramoxone. Krijnen and Boyd[11] described that the growth of the liver was inhibited by exposing to pesticides and herbicides and hepatic

Table 2. Total weight gain and feed efficiency ratio of rats fed the experimental diets

Dietary group	Total weight gain (g)						Feed efficiency ratio					
	2wks		3wks		4wks		2wks		3wks		4wks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CG	76.00^{a} (n=4)	±8.67	104.50^{a,b} (n=4)	±8.32	122.00^{a,b} (n=4)	±4.55	33.72^{a} (n=4)	±2.84	32.30^{a,b} (n=4)	±8.87	20.71^{a,b} (n=4)	±4.96
GG	40.25^{a} (n=4)	±8.73	45.75^{a,c} (n=4)	±8.46	61.75^{a,c} (n=4)	±2.74	22.14^{a} (n=4)	±8.44	16.33^{a,c} (n=4)	±6.78	15.05^{a,c} (n=4)	±2.03
GVG*			84.00^{b,c} (n=4)	±3.18	90.50^{b,c} (n=4)	±4.40			28.72^{b,c} (n=4)	±6.89	20.17^{b,c} (n=4)	±4.25

^{a,c} Mean scores are significantly different from each other in the same column ($p<0.05$)
^{b} Mean scores are not significantly different in the same column
* After feeding 18% casein + 0.04% gramoxone diet for 2 weeks, rats were fed with 3% L-ascorbic acid for 1 or 2 weeks
Others are the same as those shown in Table 1

Table 3. Liver weight of rats fed the experimental diets

Dietary group	Liver weight(g)						Liver weight/body weight(%)					
	2wks		3wks		4wks		2wks		3wks		4wks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CG	8.45^{a} (n=4)	±0.63	7.93^{a,b} (n=4)	±0.58	8.38^{a,c} (n=4)	±1.01	4.20±1.01 (n=4)		3.20±0.20 (n=4)		3.54±0.46 (n=4)	
GG	6.59^{a} (n=4)	±0.77	6.06^{a,c} (n=4)	±1.40	6.31^{a,b} (n=4)	±0.65	4.36±1.36 (n=4)		3.35±0.59 (n=4)		3.37±0.31 (n=4)	
GVG*			7.68^{b,c} (n=4)	±0.94	6.79^{b,c} (n=4)	±1.46			3.62±0.28 (n=4)		3.12 ±0.29 (n=4)	

^{a,c} Mean scores are significantly different from each other in the same column ($p<0.05$)
^{b} Mean scores are not significantly different from each other in the same column
There were no significant differences in the ratio of liver weight per body weight
Others are the same as those in Table 1 and 2

detoxification of rats was impaired.

These results suggested that vitamin C has counteracting effects on the gramoxone toxicity such as the suppression of the growth of liver.

Lipid content in the liver of rats

Hepatic lipid contents of rats fed each experimental diet was shown in Table 4.

Hepatic lipid contents seemed to increase with the extension of feeding period. Lipid contents in the liver of gramoxone group were increased significantly as compared with the control group, while lipid contents in the gramoxone +Vt C group were increased slightly as compared with the control group. Significant differences($p<0.05$) were existed in the lipid content between rats fed the control diet and the gramoxone diet for 3 or 4 weeks.

There were, however, no significant differences in the lipid content of rats fed the gramoxone diet and the gramoxone+Vt C diet. There were significant differences in the lipid contents of rats fed the control diet and the gramoxone+Vt C diet. The highest lipid content (6.23 ± 1.86) and the lowest lipid content (2.64 ± 0.69) was obtained in rats fed the gramoxone diet for 4 weeks and the control diet for 3 weeks, respectively.

The addition of gramoxone into the diets caused subsequent increase of lipid content of such treated rats, while the supplementation of vitamin C decreased lipid contents in the liver of rats. Plaa and Hewitt[29] described that the accumulation of lipid in the liver of rats resulted from the blockage of the secretion of hepatic triglyceride into the plasma, and this phenomena was induced through introduction of such foreign compounds as CCl_4, phosphorus, puromycin and tetracycline. Lombardi[30] reported that the blockage of hepatic triglyceride usage resulted from a reduction in the synthesis of VLDL in the liver of rats. According to Innami[9], fatty liver was observed in the liver of rats which were administered PCB. The materials identified were triglyceride, cholesterol and phospholipid. Choi[31] also demonstrated that total cholesterol, phospholipid and triglyceride contents of liver were increased with the introduction of prolonged alcohol into rat.

Table 4. Lipid content in the liver of rats fed the experimental diets (%)

Dietary group	2wks		3wks		4wks	
	Mean	SD	Mean	SD	Mean	SD
CG	$3.23^{b)}$ (n=4)	±0.72	$2.64^{a,c)}$ (n=4)	±0.69	$3.97^{a,c)}$ (n=4)	±0.67
GG	$4.04^{b)}$ (n=4)	±0.26	$4.44^{a,b)}$ (n=4)	±1.41	$6.23^{a,b)}$ (n=4)	±1.86
GVG			$3.82^{b,c)}$ (n=4)	±0.61	$5.40^{b,c)}$ (n=4)	±0.76

[a,c] Mean scores are significantly different from each other in the same column ($p<0.05$)

[b] Mean scores are not significantly different from each other in the same column

Others are the same as those in Table 1 and 2

Holloway and Rivers[32] reported that chronic ascorbic acid deficiency resulted in the reduced cytochrome p-450 concentration, lower cholesterol 7α-hydroxylase activity, lower bile acid turnover rate, prolonged bile acid half-life and increased plasma liver cholesterol concentrations in guinea pigs. According to Chow *et al.*[33], the intraperitoneal administration of 500mg of PCB resulted in the heightened levels of ascorbic acid in the plasma and livers, but did not affect those levels in the lungs of rats. They described that the high levels of ascorbic acid in the plasma and livers appeared to be related to the ability of the parenchymal organs to synthesize ascorbic acid for their functional need, and that this adaptive response might enable animals against further damage.

From the results of this study, it has been considered that ascorbic acid has alleviating effects on the gramoxone toxicity in rats by coping with the damage of the liver and helping the release of lipid into the plasma.

TBA values in the liver of rats

Hepatic TBA values of rats are presented in Table 5.

There were significant differences ($p<0.05$) in the hepatic TBA value of rats fed the control diet and the gramoxone diet. Significant differences ($p<0.05$) were existed in the TBA value of rats fed the gramoxone diet for and the gramoxone +Vt C diet for 4 weeks. There were, however, no significant differences in the TBA value of rats fed the control

diet and the gramoxone + Vt C diet for 3 or 4weeks. The highest TBA value ($0.31 \pm 0.05 \mu g$ MA/g) and the lowest such value ($0.11 \pm 0.02 \mu g$ MA/g) was obtained in rats fed the gramoxone diet for 3 weeks and the control diet for 2 weeks, respectively. Results of the TBA value in the liver indicated that the addition of gramoxone tended to increase hepatic TBA value in rats, while the supplementation of vitamin C tended to decrease hepatic TBA value slightly. This tendency was observed obviously in those fed the gramoxone + Vt C diet for 2 weeks.

Lipid peroxidation is designated as a chain reaction brought about by oxygen radical in polyunsaturated fatty acid[34]. According to Fred *et al.*[35], lipid peroxidation appeared to come about indirectly by OH radical formed in an interaction of superoxide and H_2O_2 in existence of ferrous ion. They reported that DNA and the cell membrane were damaged chiefly. Recknagel *et al.*[36] presented that CCl_4 had toxic effects by forming trichloromethyl free radical with mixedfunction oxidase system and cytochrome p-450, and by peroxidizing unsaturated fatty acid of phospholipid in cell membrane. According to Bus *et al.*[37] and Gage[1], the suggested mechanism of gramoxone toxicity is mediated by free radical reactions and involves the damage to cell membrane. Innami[9] reported that hepatic TBA values were increased remarkably in rats which had been administered PCB. He described that the addition of PCB into their diet resulted in the changes of fat content, formation of lipid peroxidation and increased unsaturated fatty acid levels in the liver of rats. Additionally, he suggested that this increase in unsaturated fatty acid could act as substrates for hepatic lipid peroxidation. Kim[7] demonstrated that the addition of gramoxone into diet resulted in the increase of hepatic TBA value in rats and the high protein diet had an alleviating effect on the gramoxone toxicity.

Table 5. TBA values in the liver of rats fed the experimental diets (μg MA/g)

Dietary group	2wks		3wks		4wks	
	Mean	SD	Mean	SD	Mean	SD
CG	0.11^{a} (n=4)	±0.02	$0.20^{a,d}$ (n=4)	±0.01	$0.18^{a,b}$ (n=4)	±0.02
GG	0.28^{a} (n=4)	±0.08	$0.31^{a,b}$ (n=4)	±0.05	$0.27^{a,c}$ (n=4)	±0.06
GVG*			$0.28^{b,d}$ (n=4)	±0.14	$0.18^{b,c}$ (n=4)	±0.02

[a,c] Mean scores are significantly different in the same column ($p<0.05$)
[b,d] Mean scores are not significantly different in the same column
Others are the same as those in Table 1 and 2

The addition of gramoxone tended to increase hepatic TBA value in rats irrespective of the lapse of time, while the supplementation of Vt C tended to decrease hepatic TBA value slightly. Because TBA value is related to the formation of lipid peroxidation, lipid peroxidation might start in gramoxone-treated rats. With regard to the supplementation of Vt C, hepatic TBA value of the gramoxone + Vt C diet appeared to decrease as compared with the gramoxone diet.

From the above results, it might be suggested that Vt C has alleviating effects on the gramoxone toxicity. It is, however, regrettable that this study could not explain the mechanism of alleviating effects of Vt C on the gramoxone toxicity in detail. Further researches seemed to be required.

SDS-polyacrylamide gel electrophoresis(PAGE) patterns of soluble proteins in the liver of rats

Densitograms of the SDS-PAGE of soluble proteins in the liver of rats are shown in Fig. 1, 2, 3, 4, 5, 6, 7 and 8. Seventeen protein bands were observed in the liver of rats fed the control diet for 2 or 3 weeks. Eighteen protein bands were, however, observed in the liver of rats fed the control diet for 4 weeks. Protein subunits having molecular weight larger than 68,000 D were 11 (band 1~11) and most abundant. Protein subunits larger than 43,000 D were 4 (band 12~15). Protein subunit larger than 29,000 D and smaller than 13,700 was band 16 and 17, respectively. Quantitative changes of bands were observed in the liver of rats fed the control diet with the period of feeding experiment.

Bands 1, 2, 3, 5 and 12 tended to decrease, while bands 4 and 17 tended to increase in the liver of rats fed the control diet for 3 or 4 weeks. Band of 14,300 D was observed in the liver of rats fed the control

diet for 4 weeks, which was not detected in rats fed the control diet for 2 or 3 weeks. With regard to the addition of gramoxone, protein contents decreased significantly in rats fed the gramoxone diet as compared with rats fed the control diet for 2 weeks. Band 4 was not significantly changed, and band 6 and 8 were not observed in rats fed the gramoxone + Vt C diet for 3 weeks as compared with rats fed the control diet or the gramoxone diet. Protein contents of each band decreased slightly, while protein contents of low molecular band 17 tended to increase significantly in rats fed the gramoxone + Vt C diet for 3 weeks. Protein subunit patterns of rats fed the gramoxone + Vt C diet for 4 weeks were shown to be similar with rats fed the control diet for 4 weeks, while protein contents of each band except band 1, 16 and 17 were shown to decrease. Moreover, protein contents of band 4 tended to decrease significantly.

According to Brewster *et al.*[38], the levels of many protein bands significantly flatterned in the TCDD-treated electrogram. Windmuller and Euler[39] reported that the administration of orotic acid caused to accumulate lipid in liver and to decrease the release of lipoprotein into blood. Dianzani[40] also reported that

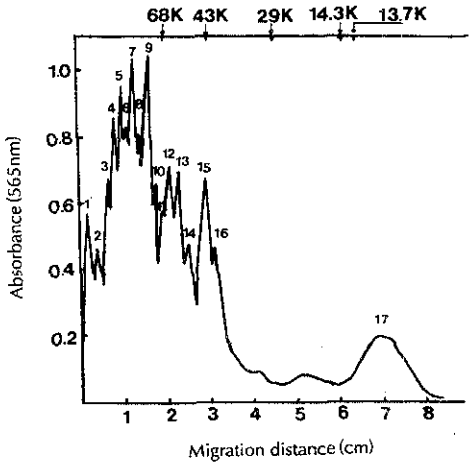

Fig. 1. Protein subunits in the liver of rats fed the control diet for 2 weeks.

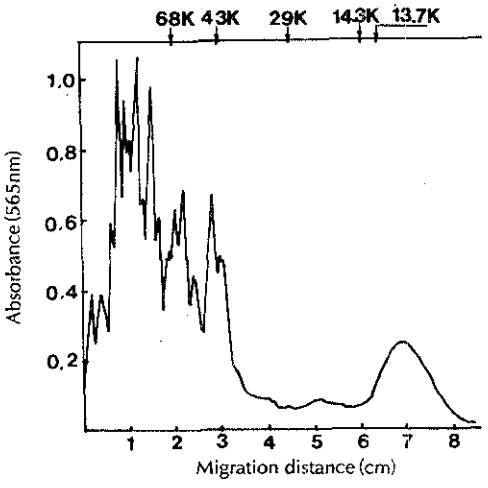

Fig. 2. Protein subunits in the liver of rats fed the control diet for 3 weeks.

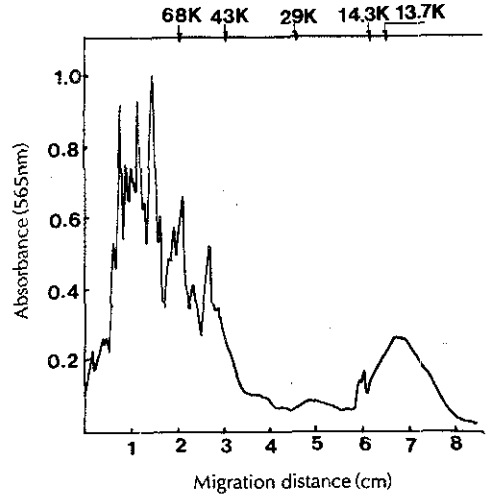

Fig. 3. Protein subunits in the liver of rats fed the control diet for 4 weeks.

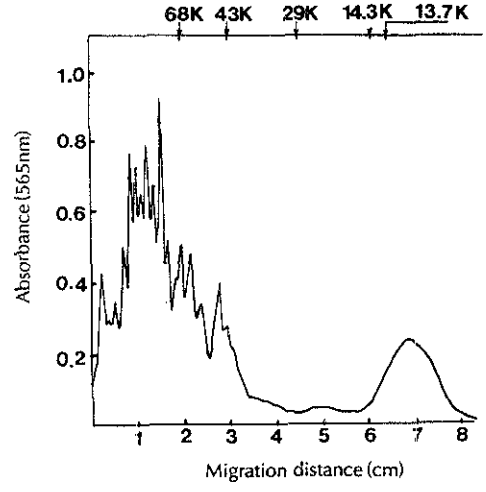

Fig. 4. Protein subunits in the liver of rats fed the gramoxone diet for 2 weeks.

the administration of orotic acid caused to increase the synthesis of total protein in liver and to decrease the union of marked amino acids and apoprotein derived from VLDL significantly.

The decreases of lipoprotein in liver were due to the defects of constituent assembling in lipoprotein by the structural changes of endoplasmic reticulum or by the changes of chemical structure and function in it, and due to the defects of releasing mechanism in lipoprorein micelle formed by the hepatic cells[40]. Lee[41] reported that the administration of ethanol resulted in the increase of protein synthesis in the liver of rat. Lieber[42] also presented that the intake of alcohol caused to increase the contents of lipid, protein and water in the cytosol of hepatic cells[42].

Judging from the results with other researches, gramoxone seeemed to decrease the protein contents in liver at the initial stage. Protein contents, however, seemed to increase respectively by the increases of the Kupffer cells and lipid contents, and the accelerations of protein metabolism in liver, and by the defects of release mechanism in lipoprotein micelle formed from the hepatic cells[40~42].

Moreover, the changes of protein bands and the

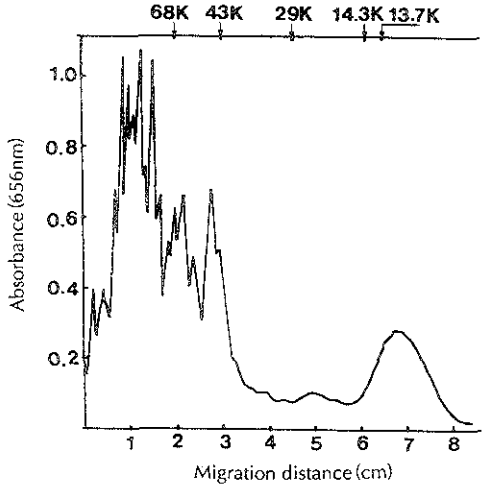

Fig. 5. **Protein subunits in the liver of rats fed the gramoxone diet for 3 weeks.**

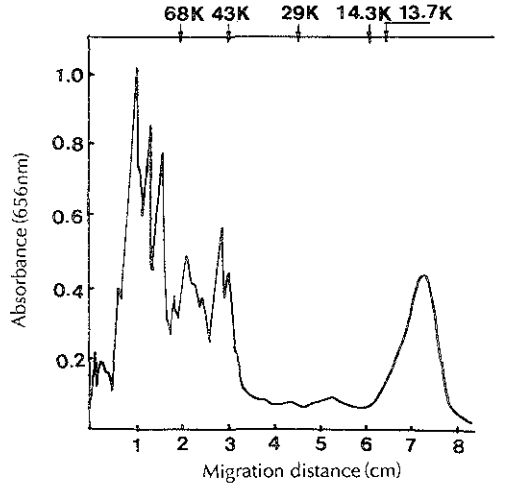

Fig. 7. **Protein subunits in the liver of rats fed the gramoxone + Vt C diet for 3 weeks.**

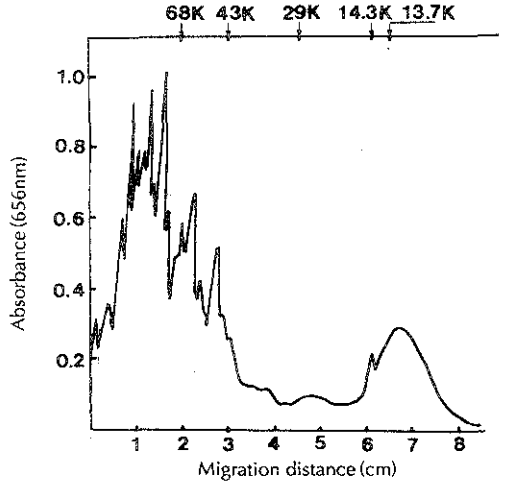

Fig. 6. **Protein subunits in the liver of rats fed the gramoxone diet for 4 weeks.**

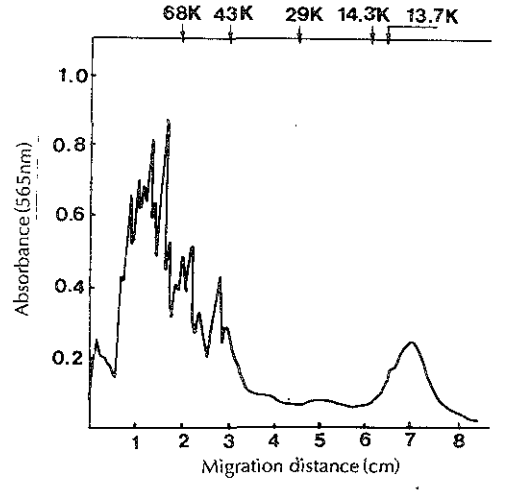

Fig. 8. **Protein subunits in the liver of rats fed the gramoxone + Vt C diet for 4 weeks.**

decreases of protein contents in the gramoxone + Vt C treated group were regarded as the results of the alleviating effects of Vt C on the gramoxone toxicity and aids of the release of the lipoprotein micelle which is the cause of the fatty liver. These results appeared to agree with the results of the alleviating effects of Vt C on the gramoxone toxicity by the decreases of lipid contents in liver and by the drop off the formation of lipid peroxidation in liver.

ACKNOWLEDGEMENT

This research was supported by a grant (911-0402 -076-1) from KOSEF.

REFERENCES

1. Gage, J. C. : The action of paraquat and diquat on the respiration of liver cell fractions. *Biochem. J.*, **109**, 757(1968)
2. Chung, B. S. : The effect of organophosphorus pesticide on oxidative phosphorylation of liver mitochondria in rats. *Pusan Med. J.*, **15**, 43(1975)
3. Murphy, S. D. : Pesticides. In "*Toxicology*" Doull, J., Klaassen, C. D. and Amdur, M. O. (eds.), Macmilan publishing Co., Inc., New York, p.357 (1980)
4. Jo, U. B. : Histochemical studies on the effect of the pyridine herbicide, gramoxone, in the activity of alkaline and acid phosphtases in the rat intestine. *J. Sci. Pusan National Univ.*, **33**, 235 (1982)
5. Kim, J. J. and Jo, U. B. : Histochemical studies on the effect of Bassa insecticide in the mucosubstances of the rat intestine. *J. Sci. Pusan National Univ.*, **31**, 167 (1981)
6. Jo, U. B. and Kim, B. S. : Histochemical study on the effect of the pyridine herbicide, gramoxone, on the mucosubstances of the goblet cells in the rat small intestine. *Kor. J. Anat.*, **20**, 299 (1987)
7. Kim, S. R. : Effect of dietary protein levels on the manifestation of gramoxone toxicity in rat. *Ph. D. Thesis.* Pusan National Univ., Pusan, Korea (1990)
8. Bus, J. S., Cagen, S. Z., Olgaard, M. and Gibson, J. E. : A mechanism of paraquat toxicity in mice and rats. *Toxicol. Appl. Pharmacol.*, **35**, 501(1976)
9. Innami, S. : Interaction between toxicity of pollutants and nutrition. *J. Jap. Soc. Food Nutr.*, **38**, 1 (1985)
10. Boyd, E. M. and Krupa, V. : Protein deficient diet and diuron toxicity. *J. Agr. Food Chem.*, **18**, 1104 (1970)
11. Krijnen, C. J. and Boyd, E. M. : The influence of diets containing from 0 to 81 percent of protein on tolerated doses of pesticides. *Comp. Gen. Pharmac.*, **2**, 373 (1971)
12. Tanaka, R., Fujisawa, S. and Nakai, K. : Study on the absorption and protein binding carbaryl, dieldrin and paraquat in rats fed on proetin diet. *J. Toxicol. Sci.*, **6**, 1 (1981)
13. Ando, M. : Studies on the effect of dietary protein and fat content upon DDT metabolism in rat liver. *J. Toxicol. Environ. Health*, **10**, 11 (1982)
14. Lipschitz, A., Bothwell, T. H., Seftel, H. C., Wapnick, A. A. and Charlton, R. W. : The role of ascorbic acid in the metabolism of storage iron. *J. Haema.*, **20**, 155 (1971)
15. Spittle, C. R. : Atherosclerosis and vitamin C. *Lancet*, **11**, 1280 (1971)
16. Machlin, L. J. : *Handbook of vitamins.* Marcel Dekler Inc., New York and Basel., p. 224 (1984)
17. Friedrich, W. : Vitamins. Walter de Gruyter, Berlin, New York, p.929 (1986)
18. Laemmli, I. K. : Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature.*, **227**, 680 (1970)
19. Weber, K. and Osborn, M. : The reliability of molecular weight determination by dodesyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.*, **244**, 4406 (1969)
20. Nie, N. H., Hu, C. H., Jenkins, J. G., Steinbrenner, K. and Bent, D. H. : *Statistical package for the social sciences.* 2nd ed., McGraw-Hill Book Co., New York,p.675(1975)
21. Cho, S. Y., Park, S. W. and Lee, S. I. : Effect of mercuric chloride on the contents of mineral nutrients in the liver and kidney of rats. *J. Korean Soc. Food Nutr.*, **13**, 22 (1984)
22. Rao, A. V. and Wong, G. K. K. : Effects of dietary protein on the pharcokinetics of butylated hydroxytoluene (BHT) in rats. *Drug Nutr. Interact.*, **2**, 69 (1983)
23. Kim, H. J., Cho, S. Y. and Park, J. M. : Effect of dietary vitamin E and protein on cadmium toxicity in rats. *J. Korean Soc. Food Nutr.*, **19**, 27(1990)
24. Fox, M. R. S., Fry, B. E., Harland, B. F., Schertel, M. E. and Weeks, C. E. : Effect of ascorbic acid on cadmium toxicity in the young coturnix. *J. Nutr.*, **101**, 1295 (1971)
25. Suzuki, T. and Yoshida, A. : Effect of dietary supplementation of iron and ascorbic acid on lead toxicity in rats. *J. Nutr.*, **109**, 982 (1979)
26. Chatterjee, K., Banerjee, S. K., Tiweari, R., Mazumdar, K., Bhattacharyya, A. and Chatterjee, G. C. : Studies on the protective effects of L-ascorbic acid in chronic chlordane toxicity. *Internat. J. Vit. Nutr. Res.*, **51**, 254 (1981)
27. Balakrishnan, G., Ramachandran, M., Banerjee, B. D. and Hussain, Q. Z. : Effect of dietary protein, dichlorophenyl-trichloroethane (DDT) and hexachlorocyclohexane(HCH) on hepatic microsomal enzyme activity in rats. *Br. J. Nutr.*, **54**, 563 (1985)
28. Wang, G. K. K. and Rao, A. V. : Effects of dietary protein on the subacute toxicity of butylated hydroxytoluene (BHT) in rats. *Drug Nutr. Interact.*, **2**, 57 (1983)

29. Plaa, G. L. and Hewitt, W. R. : Detection and evaluation of chemically induced liver injury. In *"Principle and methods of toxicology"* Hayes, A. W. (ed.), Rohm & Hass Co., Spring Hause, Pennsylvania-(1982)
30. Lombardi, B. : Fatty liver : considerations on the pathogensis of fatty liver. *Lab. Invest.*, **15**, 1 (1966)
31. Choi, K. J. : Effects of chronic feeding on the lipid contents in the rat liver and the hepatic microsomal 2-acetylaminofluorene(2-AAF) hydroxylation *in vitro*. *Ewha Med. J.*, **6**, 35 (1983)
32. Holloway, D. E. and Rivers, J. M. : Influence of chronic ascorbic acid deficiency and excessive ascorbic acid intake on bile acid metabolism and bile composition in the guinea pig. *J. Nutr.*, **111**, 412(1981)
33. Chow, C. K., Thacker, R. and Gairola, C. : Dietary selenium and levels of L-ascorbic acid in the plasma, livers and lungs of polychlorinated biphenyls-treated rats. *Internat. J. Vit. Nutr. Res.*, **551**, 279 (1981)
34. Buege, J. A. and Aust, S. D. : Microsomal lipid peroxidation. In *"Methods of enzymology"* Fleischer, S. and Packer, L. (eds.), Academic Press, New York, Vol. 53, p.302 (1978)
35. Fred, J. J., Yost, J. and Fridovich, I. : Superoxide and hydrogen peroxide in oxygen damage. *Arch. Biochem. Biophys.*, **175**, 514 (1976)
36. Recknagel, R. O., Glende, E. A. and Hruskewycz, A. M. : Chemical mechanisms in carbon tetrachloride toxicity. In *"Free radicals in biology"* Pryor, W. A. (ed.), Academic Press, New York, Vol.III, p.97 (1982)
37. Bus, J. S., Cagen, S. Z., Olgaard, M. and Gibson, J. E. : A mechanism of paraquat toxicity in mice and rats. *Toxicol. Appl. Pharmacol.*, **35**, 501 (1976)
38. Brewster, D. W., Madhukar, B. V. and Matsumura, F. : Influence of 2, 3, 7, 8-TCDD on the protein composition of the plasma membrane of hepatic cells from the rat. *Biochem. Biophys. Res. Commun.*, **107**, 68 (1982)
39. Windmueller, H. G. and Euler, L. H. : Prevention of orotic acid-induced fatty liver with allopurinol. *Proc. Soc. Exp. Biol. Med.*, **136**, 98 (1971)
40. Dianzani, M. V. : Biochemical aspects of fatty liver. In *"Biochemical mechanisms of liver injury"* Slater, T. F. (ed.), Academic Press, New York, p.45 (1978)
41. Lee, H. Y. : The effect of ethanol on the liver protein synthesis of normal and ethionine intoxicated rats. *Kyungpook Univ. Med. J.*, **11**, 329 (1970)
42. Lieber, C. S. : Alcohol and the liver : Metabolism of ethanol, metabolic effects and pathogenesis of injury. *Acta Med. Scand. Suppl.*, **703**, 1 (1985)

(Received June 18, 1993)

흰쥐 간 지질함량, 과산화지질 및 단백질양상에 미치는 제초제 Gramoxone 독성에 대한 비타민 C의 완화효과

조운복† · 김성로* · 최병태

부산대학교 생물교육과

*동래여자전문대학 식품영양과

요 약

흰쥐의 체중증가량, 식이효율, 간지질함량, 간내 과산화지질 및 단백질양상에 미치는 gramoxone독성과 비타민C의 완화효과에 대해 조사하였다. 체중증가량, 식이효율 및 간무게는 gramoxone처리군이 정상대조군에 비해 현저히 감소되었으나 gramoxone-비타민 C처리군에서 gramoxone처리군에 비해 증가되었다. 간의 지질함량과 TBA가는 정상대조군에 비해 gramoxone처리군에서 현저히 증가하였으며 gramoxone-비타민 C처리군에서 gramoxone처리군에 비해 감소되었다. 간단백질 양상은 gramoxone처리군과 정상대조군에서 별 변화가 없었고 gramoxone처리군의 2주 사육군에서 정상대조군에 비해 단백질량이 현저히 감소되었으나, gramoxone-비타민 C처리군에서 단백질양상에 변화가 왔으며 고분자량의 단백질량은 감소하고 저분자량의 단백질량은 증가하는 경향을 나타내었다.