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Effect of Gam-Roa Tea on the Metabolizing Enzyme Activity of Some Free Radical and Alcohol in Rats

Chong-Guk Yoon†, Soon-Nim Chae and Joong-Kyu Shin*

Dept. of Public Health, Keimyung University, Taegu 704-701, Korea
**Dept. of Health Science, Kyungsan University, Kyungsan 713-715, Korea*

Abstract

To investigate an effect of Gam-Roa tea on the free radical or alcohol detoxicating enzyme activities, the rats got a drink at the Gam-Roa tea instead of water for 3 months, and then the animals were sacrificed and obtained the following findings. The animals receiving Gam-Roa tea showed a decreasing tendency of hepatic xanthine oxidase activity and significantly increased content of cytochrome P-450 compared with the control. Furthermore, hepatic superoxide dismutase and glutathione S-transferase activities were also more increased in rats received Gam-Roa tea than in the control group, those receiving water. On the other hand, alcohol or aldehyde dehydrogenase activities were more increased in rats receiving Gam-Roa tea than the control. In conclusion, it is likely that the liver of rats receiving Gam-Roa tea may have the oxygen free radical or alcohol detoxication potential.

Key words: Gam-Roa tea, oxygen free radical generating, scavenging enzymes

INTRODUCTION

Gam-Roa tea is prepared from the dried leaves of Hydrangea serrate seriger var, *Thunvergii sugimoto*. This plant grows naturally in Zie-Rie mountains. In recent, those plants are cultivated at a farm in the suburb of Taegu city and produced as a tea prepared from the dried leaves on a commercial scale. Gam-Roa has known in a folk remedy or chinese medicine as therapeutic effect on the stomach lesion, bronchitis, hypertension and drug addiction. The investigation of natural products as functional foods has recently assumed a greater sense of urgency in response to the demands for good health, furthermore, therapeutic availability. It has been reported that Gam-Roa tea influences upon the antiallergic, antimicrobial action (1,2) and antidermatitis(3).

In the present study, to observe the effect of Gam-Roa tea on the detoxicating potentiality of alcohol and free radicals, the experimental rats got a drink at the Gam-Roa tea instead of water for 3 months. And then the hepatic alcohol or aldehyde dehydrogenase, xanthine oxidase, superoxide dismutase, glutathione S-transferase activities, glutathione, lipid peroxide and cytochrome P-450 contents were demonstrated.

†Corresponding author

MATERIALS AND METHODS

Animal treatment

Preparation of Gam-Roa tea: 10g dried leaves were added to 1L of water and boiled for 30 min., and then Gam-Roa tea obtained by the filtration through gauze.

Male Sprague-Dawley rats weigh about 200g had fed a commercial Jae-Il forage and experimental rats has given Gam-Roa tea instead of water for 3 months.

Animals were killed by exanguination of abdominal aorta. The liver of rats was rapidly removed and homogenized in ice-cold 0.25M sucrose. Homogenates(20% w/v) in 0.25M sucrose solution were centrifuged at 600×g for 10 min. The supernatants obtained were spun at 10,000×g for 30 min. The postmitochondrial fractions were again centrifuged at 105,000×g for 60 min., and then cytosolic fraction was utilized in the determination of xanthine oxidase(XO), superoxide dismutase(SOD), alcohol(ADH) or aldehyde dehydrogenase(ALDH), and glutathione S-transferase(GST) activities. The microsomal fraction was utilized in the assay of cytochrome P-450 content.

Biochemical analysis

Lipid peroxide(LPO) content: Lipid peroxidation in liver homogenates was estimated by measuring malondialde-

hyde formation by using the thiobarbituric acid reaction technique as described by Ohkawa et al.(4).

Protein content was assayed by the method of Lowry et al.(5).

Glutathione(GSH) content: Hepatic GSH content was determined by the color reaction of glutathione with 5,5'-dithiobis(2-nitrobenzoic acid) according to the procedure of Ellman(6).

Cytochrome P-450 content: Cytochrome P-450 content was determined by the dithionite difference method of Omura and Sato(7).

Enzyme assays

SOD activity was determined by its ability to inhibit autooxidation of hematoxylin by method of Martin et al.(8).

ADH and ALDH activity were assayed by measuring formation of NADH using substrate as ethanol, acetaldehyde respectively, coenzyme as NAD according to the procedure of Bergermeyer(9). XO activity was determined by measuring the rate of uric acid formation using xanthine as substrate by the method of Stirpe and Della Corte(10). GST activity was determined by measuring thioether using 1-chloro-2,4-dinitrobenzene and glutathione as substrate by the method of Habig et al.(11).

The statistical significance of difference between values were analyzed by student's t-test(12).

RESULTS AND DISCUSSION

One important mechanism of cell injury is induced by free radicals, particularly by activated oxygen species. It is a final common pathway of cell injury in such varied processes as chemicals and radiation injury, oxygen etc. (13,14). Such a free radical intoxication can be modified by the various physiological factor, aging, sex, diurnal cycles and nutritional conditions(15). Especially it seems to be important to elucidate the nutritional or medicinal herbs effect on the free radical induced injury.

All the more the obervation of bioactive natural products in a functional food has, in recent years, assumed a greater sense of urgency in response to the free radical induced injury and these bioactive substances can be done the protective action on the cell injury(16).

Furthermore, various tea brands prepared from the functional taste food are producing on the commercial products. Here we demonstrate effect of Gam-Roa tea on the detoxication latency on the free radical or alcohol in rats.

The changes of body weight, liver weight per body weight and hepatic lipid peroxide content

Fig. 1 showed that changes of the body weight in the animals receiving Gam-Roa tea appeared to be similiar with the control group.

As shown in Table 1, the rats receiving a Gam-Roa tea showed the similiar value of liver weight per body weight(%), LPO contents and the levels of serum ALT activity with the control group. These results suggested that Gam-Roa tea used in the present experimental condition may not render liver injury.

Effect of Gam-Roa tea on hepatic cytochrome P-450 content and XO activity

It is well known the cytochrome P-450(17) and XO

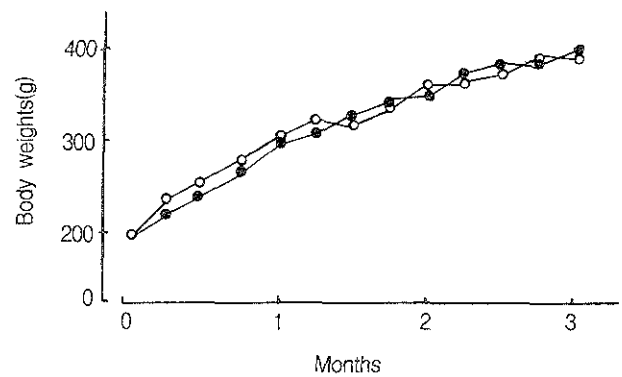

Fig. 1. Three month weight gains in rats fed Gam-Roa tea or water.
●—●: *Control groups*
○—○: Groups fed Gam-Roa tea

Table 1. Comparison of liver wt./body wt.(%), hepatic lipid peroxide(LPO) and serum alanine aminotransferase(ALT) in group fed a tea from Gam-Roa tea with those in control

Groups	Liver wt./body wt.(%)	LPO[1)]	Serum ALT[2)]
Control	1.94±0.07	100.00±8.40	25.00±0.20
Gam-Roa tea	2.09±0.06	97.00±7.51	27.14±0.15***

Each value represents the mean±S.E of 5 rats
[1)]Relative content to the control(%), [2)]Karmen unit/ml of serum
Significantly different from the control group(***$p<0.001$)

Table 2. Comparison of hepatic oxygen free radical generating enzymes activities in group fed a tea from Gam-Roa tea with those in control

Groups	XO[1)]	Type conversion of XO[2)]	Cytochrome P-450[3)]
Control	2.50±0.21	20.50±1.72	0.35±0.02
Gam-Roa tea	2.13±0.01	15.71±1.24	0.45±0.03*

Other abbreviations are the same as in Table 1
[1)]nmoles uric acid/mg protein/min, [2)]O/D+O×100(%),
[3)]μmoles/mg protein
Significantly different from the control group(*$p<0.05$)

Table 3. Comparison of hepatic oxygen free radical scavenging enzymes activities and GSH content in fed a tea from Gam-Roa tea with those in control

Groups	SOD[1)]	GSH[2)]	GST[3)]
Control	7.81 ± 2.50	3.69±0.17	720.50±49.82
Gam-Roa tea	23.553±10.81	3.68±0.34	871.95±67.26

Other abbreviations are the same as in Table 1
[1)]Unit#/mg protein(#: 50% inhibition of autooxidation of hematoxylin)
[2)]μmoles/g of tissue
[3)]2,4-Dinitrobenzene-glutathione conjugate nmoles/min/mg protein

Table 4. Comparison of hepatic alcohol metabolizing enzymes activities in fed a tea from Gam-Roa tea with those in control

Groups	ADH	ALDH
Control	4.16±0.28	3.80±0.25
Gam-Roa tea	4.39±0.01	5.29±0.68

Other abbreviations are the same as in Table 1
Unit: nmoles NADH/mg protein/min

(18) are responsible for the free radical formation. Especially xanthine oxidase in mammals is two forms; a dehydrogenase(type D), which used NAD^+ as an electron acceptor, and an oxidase(type O) with O_2 as an electron acceptor. The XO of rat liver supernatant is chiefly an NAD^+-dependent dehydrogenase as its native form(9). Yoon and Huh(19) reported that the type conversion of XO (type D→O) was demonstrated on rat liver by CCl_4 treatment. Furthermore MecCord and Roy(20) observed that type D would be converted into oxidase form of the enzyme in response to cell injury during hypoxia. In this study, the activity of liver XO(18) known as an enzyme producing the oxygen free radical was slightly lower in the rats receiving a tea from Gam-Roa tea than in the control. Especially whileas the type conversion rate of XO in fed Gam-Roa tea decreased at 23% compared to the control, hepatic cytochrome P-450 content was in those fed a tea from Gam-Roa tea increased at 28% as compared to the control(Table 2). These results indicated that the Gam-Roa tea may led to inhibition of oxygen free radical induction.

Hepatic SOD, GST activities and GSH content

Table 3 showed that glutathione, its conjugating enzyme, GST which acts as free radical scavenger(21) and SOD were demonstrated in liver of rats. The animal receiving Gam-Roa tea showed 3 fold increased activity of hepatic SOD(22) known as an oxygen free radical scavenging enzyme compared with the control. And the rats receiving Gam-Roa tea showed similiar value of hepatic glutathione contents, but 21% increased activity of GST compared with the control.

These results suggested that the Gam Roa tea may have a bioactive availability for the inhibition of oxygen free radical and it activate some xenobiotics.

The activity of alcohol metabolizing enzymes

It is well known that aldehydes are toxic substance which is detoxicated by the aldehyde dehydrogenase(23).

Alcohol dehydrogenase activity in rats received Gam-Roa tea was similiar with the control, but aldehyde dehydrogenase activity was increased to 39% in group fed Gam-Roa tea(Table 4).

Therefore these results indicate that the detoxication potential of alcohol may be in the liver of rats receiving Gam-Roa tea..

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