Efficacy and Safety of Health Foods: Use and Regulation

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There are various kinds of Foods in category such as Dietary Foods, Food Expander, Health Foods, Functional Foods, foe example. The Health Foods are hard to classify clearly from Dietary Foods or Functional Foods due to application for the health purpose. However, general toxicities such as immunogenicity acute, semi-acute and chronic toxicities should be checked before on market as Health Foods more strictly than general foods.

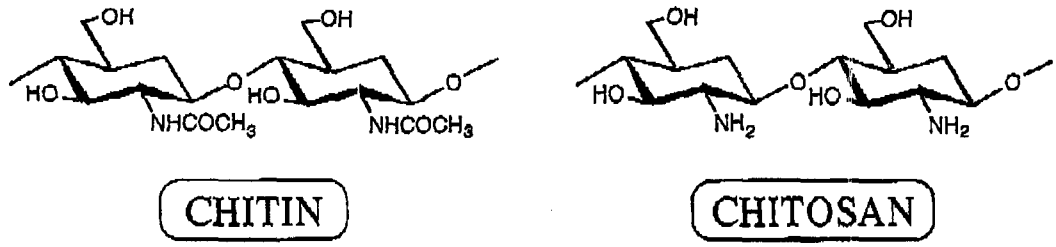


Figure 1. Chemical structures of chitin and chitosan

In the case of Chitin and chitosan which are abundant in nature as supporting mucopolysaccharide of Crustaceans. Chitin is consisted of N-acetyl-glucosamine(GlcNAc) and chitosan is the polymer of glucosamine(GlcN) as shown in Figure 1. Chitin and chitosan are indigestible polysuccharide in stomach and intestine system of human kind, because human doesn't contain bacteria in intestineto hydrolyze chitin and chitosan due to β-1,4 glycoside linkage like cellulose. Chitin is well known as nontoxic polysaccharide including low immunogenicity GlcNAc and GlcN, since chitin and chitosan are hydrolyzed into their oligomers, GlcNAc and GlcN in animal body when they are administrated by hypodermic, subcutaneous, intraperitpneal, intramuscle and intravenous injections as shown in Figure 2.

The reason why they are digestible in animal body including human kind is the presence of hydrolyzing enzymes in tears, saliva and lymph who are attached to protect from bacterial infection originally. The mechanism of protection against bacterial infection is the break down the cell wall by hydrolyzing enzyme such as lysozyme, chitinases or chitosanases. But the rate of chitosan hydrolysis is usally lower than that of chitin probably due to chitosan hydrolysis by subfunction of some enzyme in animal body. We have checked the immunogenicity of chitosan through the mouse peritoneal macrophage activation which induces secretion of cytokines and other immunogenic factors as shown in Table 1.

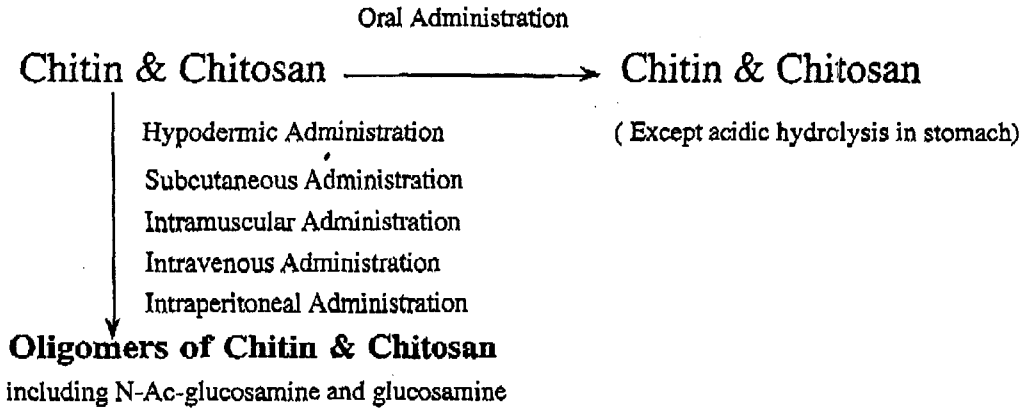


Figure 2. Hydrolyses of chitin and chitosan in human body.

Table 1. Immunological properties of chitosan in mouse.

Activity	DAC-70	CM-chitin	BCG-CWS	MDP
Adjuvant activity				
1. DTH				
ABA-N-acetltyrosine	+			+
2. CMC				
P815 (in vivo)	+	−	+	−
(in vitro)	±		+	+
3. Ab production				
BαA (in vivo)	+	−		+
DNP hapten (in vitro)	+	−	+	
4. Macrophage activation				
cytolysis (in vivo)	+	+	+	
cytostasis (in vivo)	+	+	+	
H_2O_2 release (in vivo)	+	+	+	
(in vitro)	−	±		+
5. Cytokine production				
IL-1 (in vitro)	+	−	+	+
IL-2 (in vitro)	−		+	
CSF (in vivo)	+		+	
MAF (in vitro)	−	−	+	
TNF (in vivo)	−		+	
IFN (in vivo)	+	−	+	
(in vitro)	−	−	+	
Mitogenic activity (in vitro)	−	±	+	−

Antitumor activity				
supression of Meth-A	+	−	+	−
Antiinfectious activity				
E. coli	+	−	+	+
Sendai virus	+	−		±

According to our investigation, chitosan is an immunoadjuvant of medium-class such as lentinan from brown mashrooms. The characteristics of chitosan are listed in Scheme 1. As shown in Scheme 1, chitosan sdsorbes bile acid or flocculates organic materials in intestine including E. coli to carry out it as faces as listed in Table 2. The serum levels of total choledtrols and tri-glycerol are suppressed siginificantly by the addition of chitosan, when high cholesterol food is administrated orally to rabbits as shown in Table 3. The suppression of their contents in liver were also observed by the addition of chitosan to high cholesterol food. As chitosan is also reported to adsorb endotoxines which are produced by the destruction of gram negative bacteria and highly toxic lipoprotein, the removal of endotoxin is requested prior to administration even if oral.

Although chitosan doesn't be susceptible for hydrolyzing enzymes in stomach and intestine system of human, some amounts of chitosan oligomers are supposed to be produced during stay in stomack of human due to low pH. As the chitosan oligomers are permeable through intestinal wall, toxicities of chitosan oligomer are requested strongly before application as health foods, because chitosan is shown to be immunoadjuvant and induced fibroblast formation. However, chitosan oligomers permeated through intestinal wall are expected to enhance the cell activity in human body, since chitosan oligomers are reported to stimulate the activity of bacteria such as E. coli and others as shown in Figure 3 in which high molecular weight chitosan inhibits E. coli activity specifically and low molecular one accelerates E. coli activity. When fluorescent labelled chitosans of high molecular weight and low molecular weight were incubated with E. coli, high molecular weight chitosan inhibited E. coli activity through stacking out side of cell wall and low molecular weight one permeated into the cell to stimulate E, coli activity significantly in which accumulations of fluorescent were detected by conforcal laser microscope. The accumulations of chitin oligomer were observed in the spleen by oral administration for mouse and that of bone marrow by intravenous administration of ^{14}C labelled chitin derivatives. Although chitin and chitosan are hard to be hydrolyzed in stomach and intestine of human, chitosan ligomers are suggested to be accumulated in the spleen by oral administration of chitosan oligomers. But chitosan oligomers are unable to administrate intravenously due to blood clottable property of chitosan. These results are likely to tell us the advantage of chitosan oligomers on the regulation of physiological activity of animal including human. However, chitosan oligomers should be classified clearly as health foods under the strict regulation by law, because toxicities including side effect, immunogenicity and etc. are still not satisfied at the present time.

Characteristics of Chitosan --- Cationic polymer(pKa 6.4)

Flocculation of organic materials （Especially of anionic compounds)

Ionic bindings of various metals (Especially heavy metals)

Immunoadjuvant activity (Intraperitoneal administration)

Induction of fibroblast formation

Blood coagulant

Antimicrobial activity against various bacteria and virus selectively independent to gram strain. **Effective for** (E. coli（大腸菌), Enteropathogenic E.coli,（ETEC, 毒素原性大腸菌), Staphylococcus aureus（黄色ブドウ球菌) etc.)

Less effective for Salmonella typhimurium（ネズミチフス菌), Bacillus cereus（セレウス菌)

LD_{50} on the oral administration to rat is 16g/Kg of body weight, less than 1g/Kg of body weight for chickin. less than 4.5mg/Kg of rabbit weight on the intravenous injection of chitosan oligomer.

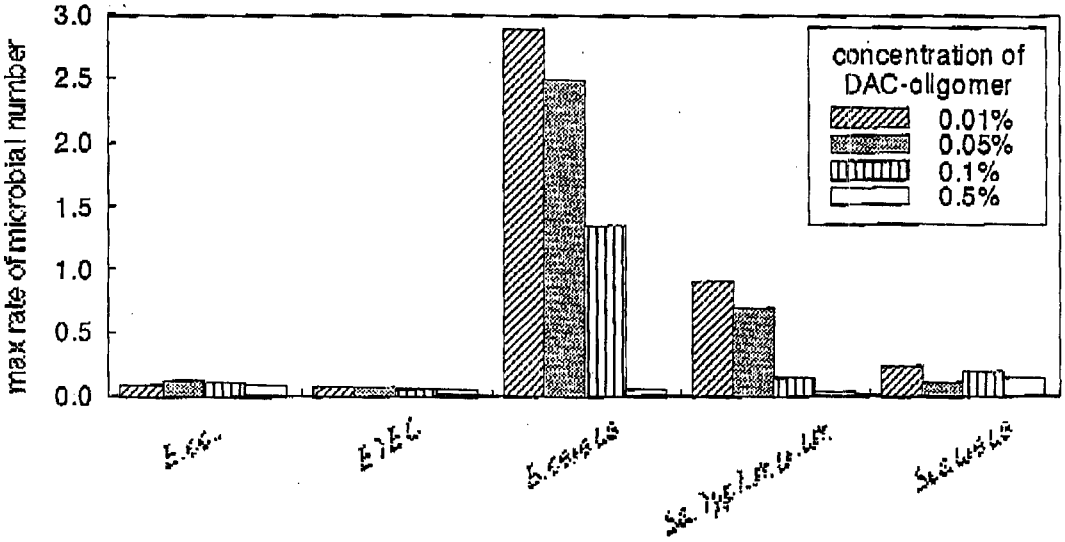
Scheme 1. Characteristics of chitosan

Table 2. Dependence of Feed composition on Cholesterol and Bile Acid Contents in the Feces of Rabbits.(S. Hirano et.al. Chitin Derivatives in Life Sci., eds. S. Tokura & I. Azuma, p. 115-120, Sapporo(1992).

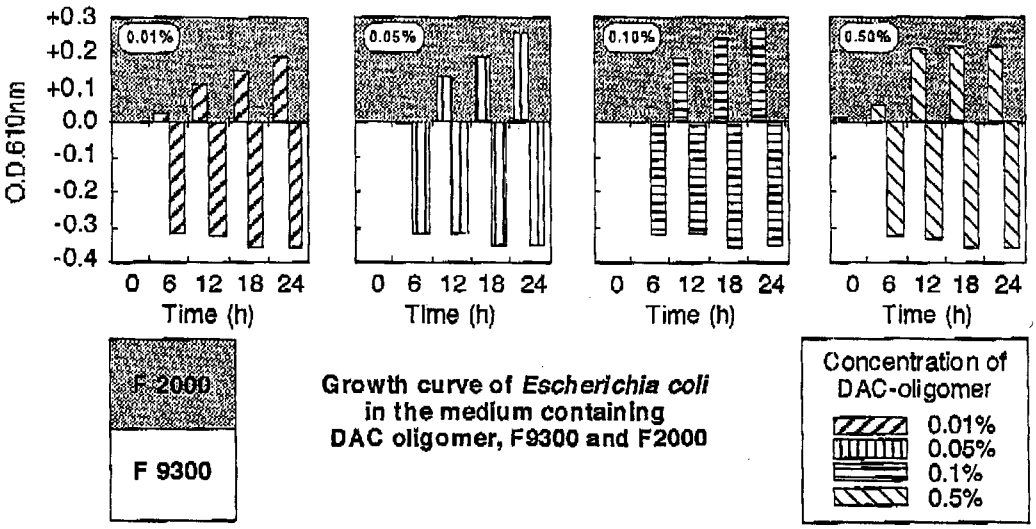
Sterol and Bile Acid (ug/100mg of dried feces)

	Baisc Feed	Basic Feed + Cholesterol (0.9%)	Basic Feed + Cholesterol (0.9%) + 2% of Chitosan
Bile Acids			
Cholic Acid	54	68+10	340+50
Ketolithocholic Acid	174+40	170+40	66+25
Deoxycholic Acid	86+7	90+33	114+30
Lithocholic Acid	47+5	49+20	63+12
Others	300+60	280+50	340+60
Total	661	657	923
Sterols			
Coprostanol	1210+70	1200+90	1320+35
Coresterol	220+120	320+40	470+38
Total	1430	1520	1790
Overall	2091	2177	2713

The medical evaluation of chitosan oligomers is still under investigation including acccumulation in organs and metabolism of chitosan oligomers. Chitosan oligomers would be evaluated like as oriental medicine when medicinal effects are found on chitosan oligomers. But medicinal effects are quite different category from health foods.



The rate of microbial numbers determined by measurement of the optical density of several strains



Growth curve of *Escherichia coli* in the medium containing DAC oligomer, F9300 and F2000

Figure 3. Antimicrobial activity by chitosan of different molecular weight.