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REVIEW ARTICLE

Investigation of nutriactive phytochemical – gamma-oryzanol in experimental animal models

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Summary

Gamma-oryzanol (GO) is an abundant dietary antioxidant that is considered to have beneficial effects in cardiovascular disease, cancer and diabetes. Other potential properties of GO include inhibition of gastric acid secretion and decreased post-exercise muscle fatigue. GO is a unique mixture of triterpene alcohol and sterol ferulates present in rice bran oil, a byproduct of rice processing. GO has been studied by many researchers over the last three decades. In particular, the utility of GO supplementation has been documented in numerous animal models. A large variety of species was examined, and various experimental methodologies and targets were applied. The aim of this study was to summarize the body of research on GO supplementation in animals and to examine possible mechanisms of GO action. Furthermore, while the safety of GO supplementation in animals has been well documented, studies demonstrating pharmacokinetics, pharmacodynamics and efficiency are less clear. The observed differences in these findings are also discussed.

Keywords antioxidant, atherosclerosis, diabetes, cancer, absorption, performance

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Introduction

Gamma-oryzanol (GO) is a nutriactive phytochemical naturally occurring in crude rice bran oil (*Oryza Sativa* L.) in concentrations ranging from 1.5% to 3%. It can also be found in lesser amounts in barley and maize. Rice bran is a byproduct of rice milling. Crude rice bran oil (RBO) can be extracted from rice bran by solvent extraction using food grade n-hexane or superficial fluid extraction technology. To meet the specifications of edible oil, crude RBO is subjected to either a chemical or physical refining process resulting in loss of most of the unsaponifiable component containing GO (Patel and Naik, 2004).

According to Patel and Naik (2004), GO can be extracted from rice bran using several different methods, but superficial fluid extraction is the most efficient (Godber and Xu, 2000). Moreover, GO can be simultaneously separated and quantified from crude RBO by high-performance liquid chromatography (HPLC) (Xu and Godber, 1999).

Initially, GO was thought to be a single compound, but a study employing HPLC (Xu and Godber, 1999) revealed that it is a mixture of at least 10 phytosteryl ferulates (Fig. 1). Cycloartenyl ferulate, 24-methylenecycloartenyl ferulate and campesteryl ferulate have been identified as the major components, accounting for approximately 80% of GO (Fig. 2).

Gamma-oryzanol has been reported to possess a wide range of potential therapeutically useful biological activities, including reduction in cholesterol levels, inhibition of platelet aggregation and improvement of plasma lipid pattern. Other suggested properties of GO include: antioxidant, anticancerogenic, antidiabetic, antiulcerogenic, neuroprotective and immunomodulating action. Moreover, GO is a popular ergogenic aid that has been proved to be safe with no major side effects reported in animals and humans; therefore, it is registered in United States Food and Drug Administration system (UNII SST9XCL51M) (http://fdasis.nlm.nih.gov/srs/). GO emulsion can be purchased for supplementing diets of humans, dogs and horses (Ostaszewski et al., 2012).

While some of the potential applications of this supplement described in the literature address humans, the majority of scientific reports involve *in vitro* and *in vivo* animal studies. Thus, the objective of this article is to comprehensively review recent findings about the potential use of GO as a nutriceutical with a special emphasis on animal experimental models.

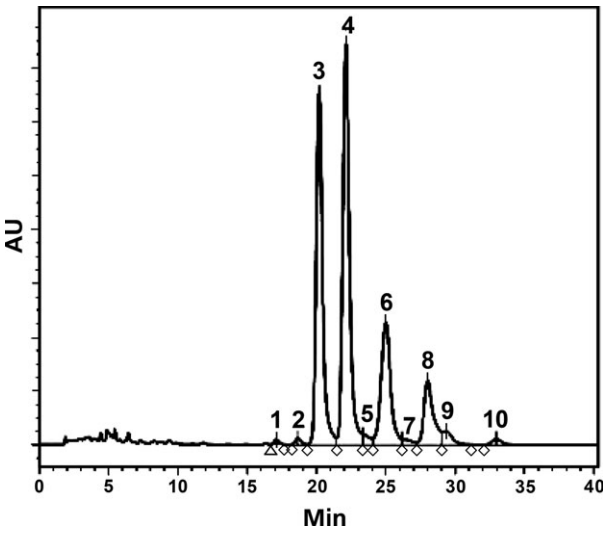

Fig. 1 Representative chromatographic profile of GO as determined by HPLC coupled with a photodiode array UV detector at 325 nm. Identified compounds in GO include the following: (1) Δ7-stigmasteryl ferulate; (2) stigmasteryl ferulate, (3) cycloartenyl ferulate; (4) 24-methylenecycloartenyl ferulate; (5) Δ7-campestenyl ferulate; (8) sitosteryl ferulate; (9) campestanyl ferulate; and (10) sitostanyl ferulate. Adapted from: Mäkynen et al. (2012).

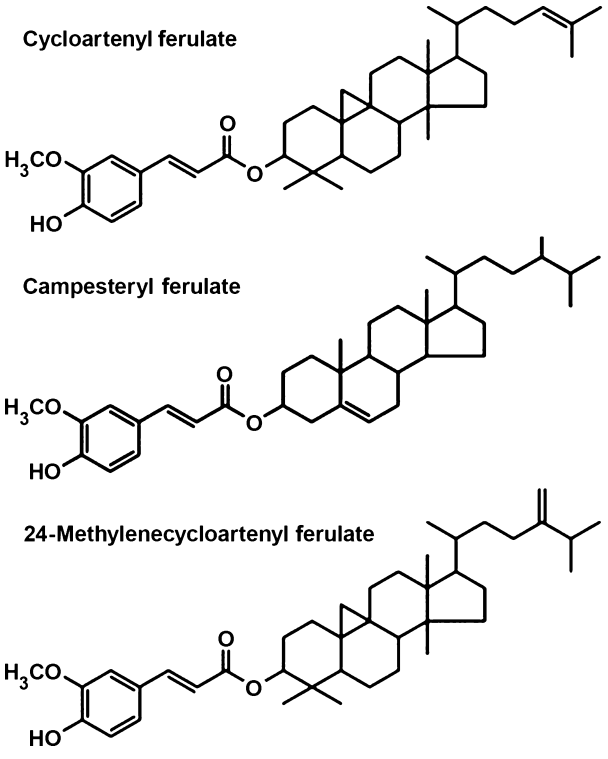

Fig. 2 Chemical structure of major components of GO: cycloartenyl ferulate, 24-methylenecycloartenyl ferulate and campesteryl ferulate.

Gamma-oryzanol digestibility

Gamma-oryzanol occurs as a mixture of conjugated phytosterols also called hydroxycinnamate esters (Moreau and Hicks, 2004). Very little is known about how phytosterol conjugates are hydrolyzed and metabolized during digestion.

In situ experiments indicated that 14C-labelled GO was partially hydrolyzed in the intestine after oral administration in male rats; 89.4% of the labelled GO remained in the luminal fluid unaffected by stomach, duodenum or jejunum digestive enzymes. More than 80% of the GO transferred into the mesenteric vein and then into portal vein remained intact, and 10 to 20% of the absorbed dose was metabolized in the intestinal tissues to yield ferulic acid. Radioactivity analysis conducted in the first 72 h after oral administration showed that 9.8% of the dose was excreted in the urine (Fujiwara et al., 1983). Moreover, Tsushimoto et al. (1991) noted that in humans, maximal plasma concentration of GO after an oral therapeutic dose of 300 mg was approximately 40 ng/ml.

Huang (2003) examined GO uptake in the *in vitro* model. A 2-h-long incubation of 0.1 mM GO micellar solution with human large intestinal cells did not show any GO absorption; however, physiological absorption of cholesterol takes place in the small intestine. Moreover, the author investigated the metabolic fate of GO by simulating peptic and pancreatic digestion by employing HPLC analysis to determine remaining GO concentrations, and gas chromatography coupled with mass spectrometry to identify digestion products. The results showed that GO concentration in a crystalline and micellar solution decreased after peptic and pancreatic digestion. The micellar solution was more susceptible to enzyme activity. The subsequent experiment demonstrated that cholesterol esterase was responsible, in a concentration-dependent manner, for pancreatic digestion. Apparently, chromatograms revealed that campesteryl ferulate and sitosteryl ferulate were most reactive to the digestive enzymes. This is in agreement with another study demonstrating that sitosteryl ferulate is hydrolyzed to a greater extent (55–85% cholesterol esterase, 50% pancreatin) than GO (35–55% cholesterol esterase, 0% pancreatin) (Moreau and Hicks, 2004). Likewise, Miller et al. (2004) performed similar incubations of GO and demonstrated that sitosteryl and campesteryl ferulates were hydrolysed by cholesterol esterase, whereas 24-methylcycloartnyl ferulate was not subjected to this process.

Campesteryl ferulate and sitosteryl ferulate are called desmethyl phytosterols due to the absence of

the dimethyl and methyl group connected with the sterol ring (Fig. 1). Their chemical structure is similar to cholesterol and that feature probably determines their susceptibility to digestive enzymes (Miller et al., 2004).

The presented results confirm that GO should be supplemented in micellar solution (emulsion) because it allows better distribution and more efficient digestion. The growing popularity of GO as a multipurpose phytosterol requires more *in vivo* studies addressing its digestibility and pharmacokinetics. Furthermore, no data has been collected regarding interspecies differences in GO digestibility (the only experiment was conducted in rats) and concerning GO biotransformation by colon microflora.

Safety of gamma-oryzanol supplementation

Preliminary acute oral intake studies showed very high LD_{50} values, that is 25 g/kg body weight (BW) in male and female mice and rats (Tsushimoto et al., 1991). The safety of GO consumption was evaluated in rats receiving a diet containing 10% RBO for 3 months (Rukmini, 1988). Control animals received peanut oil in which fatty acid composition is very similar to RBO except for the unsaponifiable matter, which is higher in RBO. The RBO effect on growth performance, including weight gain and feed efficiency, mineral balance, fat absorption and serum haematology, was not significantly different between the groups. The only exception was lower serum and liver lipids levels in the RBO group. Moreover, the toxicological effect of rice bran oil on reproductive performance was evaluated for two matings and three generations. The results indicated that no abnormalities were found in the percentage of conception, birthweight, litter size, weaning weight, pre-weaning mortality and the number of days taken to deliver from the date of introduction for mating.

Furthermore, the short-term safety of GO was assessed using the Rec assay (bacterial DNA repair test), the Ames test (bacterial reverse mutation test), the rat bone marrow chromosome aberration test and the metabolic cooperation inhibition test using Chinese hamster V79 cells (Tsushimoto et al., 1991). GO showed negative responses to all these tests.

Additionally, the potential carcinogenic effect of GO was studied by feeding mice a diet containing GO up to 2 g/kg BW/day for 78 weeks (Tamagawa et al., 1992b) and likewise, in rats during a 2-year-long administration (Tamagawa et al., 1992a). No treatment-related change was found in general condition, food consumption, mortality, organ weight and haematology. Histopathological examination demonstrated that the tumour incidents were not significantly different between the experimental and the control groups.

In summary, studies suggest that GO and RBO are not carcinogenic and are safe for animals. Moreover, GO has been approved for sale in many countries and there have been no acute or chronic side effects of GO reported. The evidence of its beneficial effects in humans is increasing and the market is growing.

Experimental doses of gamma-oryzanol

Earlier reports in animals are very inconsistent in terms of GO doses, methods of administration, the length of supplementation and type of solvent. The dosage range utilized for various animal models both *in vivo* and *in vitro* is displayed in Tables 1 and 2, respectively, in order to make them easily understood and useful for further research. The studies in which they were applied are discussed further below.

Gamma-oryzanol supplementation in preventing cardiovascular disease

High serum cholesterol is a major cause of coronary atherosclerosis, as it leads to the development of chronic heart disease. To reduce the burden of coronary atherosclerosis in society, serum cholesterol concentrations must be kept within a physiological range. There is plentiful research evidence showing that oils containing saturated fatty acids raise serum total cholesterol (TC), especially low-density lipoprotein cholesterol (LDL-C), whereas those enriched in unsaturated fatty acids lower LDL-C. In the 1950s, several studies revealed cholesterol lowering properties of plant sterols also known as unsaponifiables (Peterson, 1951; Pollak, 1953a,b; Best et al., 1954). The RBO antihyperlipidaemic property in humans was reported for the first time by Suzuki and Oshima in 1970. Since the fatty acid profile of RBO is different from other popular vegetable oils with hypocholesterolaemic potential, its abilities have been attributed to its unusually high content of unsaponifiable fraction: gamma-oryzanol (Sharma and Rukmini, 1987), which constitute up to 3% of RBO (Nicolosi, 1994).

Numerous studies in rodents have been performed to demonstrate GO's effect on the circulating levels of lipoproteins and cholesterol. In the first scientific result published in English (earlier: Kuzuya et al, 1980 in Japanese), Shinomiya et al. (1983) described an experiment in which they divided rats into four groups, three of which (II,III,IV) received a high

Table 1 Chronological list of gamma-oryzanol (GO) dosages used in *in vivo* animal studies

Experiment	Subject	Dose (Solvent)	Way of supplementation	Duration of supplementation	Efficiency/finding
Itaya and Kiyonaga (1976)	Rat	1–100 mg/kg BW	s.c.	5 days	Reduced ulcer index in both adrenalectomized and control rats.
Itaya et al. (1976)	Rat	100 mg/kg BW	s.c.	Up to 10 days	Anti-ulcer and gastrin lowering effect.
Itaya et al. (1977)	Rat	100 mg/kg BW	s.c.	5 days	Anti-ulcer and gastrin lowering effect, increment of catecholamines in rat brain.
Mizuta and Itaya (1978)	Rat	100 mg/kg BW	s.c.	5 days	Depressed gastric secretion stimulated by insulin or 2-deoxy-D-glucose.
Fujiwara et al. (1983)	Rat	50 mg/kg BW	p.o. (by gavage)	Single administration	Partial hydrolysis of 14C-labelled GO in the intestine after oral administration in male rats.
Shinomiya et al. (1983)	Rat	0.5%, 2% alone with high cholesterol diet	p.o. no additional data	Up to 12 weeks	Decreased TC, LDL and VLDL-cholesterol concentrations in the plasma, reduced activity of cholesterol acyltransferase activity in the aorta of supplemented animals.
Ichimaru et al. (1984)	Mice	100–500 mg/kg BW	p.o.	Twice in 6 h intervals	Anti-ulcerative action on gastric lesions and a suppressive action on intestinal motility.
Nakayama et al. (1987)	Rat	100, 500, 1000 mg/kg BW	p.o. (by gavage)	12 days	Decreased hyperlipidaemia in HCD treated rats
Sakamoto et al. (1987)	Rat	100 mg/kg BW; 10 mg/kg BW (Carboxymethylcellulose, propylparaben, polyvinyl alcohol)	p.o., i.v., no further details given	12 days	No hypolipidaemic effect after oral administration; intravenous administrations significantly inhibited the increases in serum TC, phospholipid and free cholesterol levels.
Seetharamaiah and Chandrasekhara (1990)	Rat	100 mg/animal 0.5% (peanut oil)	p.o.	Single dose/7 weeks	Decreased cholesterol absorption, increased bile secretion.
Seetharamaiah et al. (1990)	Rat	0.5%	p.o.	7 weeks	Reduced ADP and collagen-induced platelet aggregation after feeding with HCD diet
Hiramatsu et al. (1990)	Rabbit	1%	p.o.	10 weeks	Little or no preventive effects on atherosclerosis in rabbits.
Hirose et al. (1991)	Rat	1%	p.o.	32 weeks	Increased incidence of multiplicity of lung tumours in a multi-organ carcinogenesis model.
Tamagawa et al. (1992a)	Mice	200, 600 or 2000 mg/kg BW	p.o.	78 weeks	No observed treatment-related changes in general condition, food consumption, mortality, organ weight or haematology.
Tamagawa et al. (1992b)	Rat	200, 600 or 2000 mg/kg BW	p.o.	2 years	No observed treatment-related changes in general condition, food consumption, mortality, organ weight or haematology.
Rong et al. (1997)	Hamster	1% (coconut oil)	p.o. (*ad libitum*)	7 weeks/10 weeks	Cholesterol lowering action and reduction in aortic fatty streaks formation in HCD administered animals.

Table 1 (Continued)

Experiment	Subject	Dose (Solvent)	Way of supplementation	Duration of supplementation	Efficiency/finding
Hirose et al. (1998)	Rat	1%	p.o.	32 weeks	Increase incidence of multiplicity of lung tumours in a multi-organ carcinogenesis model. No significant differences in the final incidences and multiplicities of mammary tumours.
Lee et al. (2004)	Mice	0.2%	p.o.	8 weeks	Decreased serum glucose levels in diabetic mice.
Wilson et al. (2007)	Hamster	0.5% along with HCD	p.o. (*ad libitum*)	10 weeks	Anti-atherogenic effect.
Islam et al. (2008)	Mice	100 mg/kg BW; 50 mg/kg BW (0.01% Tween-20, 0.5% carboxymethylcellulose)	s.c.; p.o.	18 days along with dextran sulphate sodium	Anti-inflammatory effect in dextran sulphate sodium-induced colitis.
Chotimarkorn and Ushio (2008)	Mice	0.025 mmol per animal	p.o.	30 days	Prevention of ethanol-induced liver injury.
Ohara et al. (2009)	Mice	3.125; 12.5; 25 μmol (beef tallow)	p.o.	Single administration of 0.5 ml of GO solution	Increased serum adiponectin secretion in physiological condition and after LPS-induced NF$\kappa\beta$ signalling activation.
Ismail et al. (2010)	Rat	100 mg/kg BW (1% Tween 80)	p.o. (by gavage)	5 weeks	Antioxidative effect via regulation of the expression of antioxidant and oxidative stress related genes.
Mösseler et al. (2010)	Horse	2 g per animal	p.o.	31 days	No observed effect on serum and urine testosterone levels.
Son et al. (2011)	Mice	0.5% along with HCD	p.o. (*ad libitum*)	7 weeks	Reduced risk of high-fat diet-induced hyperglycaemia via regulation of insulin secretion and hepatic glucose-regulating enzyme activities.
Kim et al. (2012)	Mice	0.2%; 0.5%; 1%	p.o. (*ad libitum*)	4 weeks	Tumour regression expressed by induction of natural killer cells activity, activation of macrophages and inhibition of angiogenesis.
Ostaszewski et al. (2012)	Horse	3 g/per animal (rice bran oil)	p.o	16 weeks	Decrease in muscle fatigue parameters.
Ghatak and Panchal (2012a)	Rat	50, 100 mg/kg BW (4% Tween 80)	p.o.	21 days followed by Trition WR-1339 injection	Increased cell mediated immunity, increase in delayed-type hypersensitivity reaction, amelioration of cyclophosphamide-induced myelosuppression
Ghatak and Panchal (2012b)	Rat	25, 50, 100 mg/kg BW (4% Tween 80)	p.o.	28 days/10 days along with c yclophosphamide	Significant decrease in the levels of serum cholesterol, triacylglycerides, LDL, VLDL and a significant increase in the level of serum HDL and hepatic antioxidant enzymes.
Ghatak and Panchal (2012c)	Rat	50, 100 mg/kg BW (4% Tween 80)	p.o.	Single administration	Decrease in the extent of lipid peroxidation and increased levels of enzymatic antioxidants such as superoxide dismutase and reduced glutathione in the liver.

Table 1 (Continued)

Experiment	Subject	Dose (Solvent)	Way of supplementation	Duration of supplementation	Efficiency/finding
Ghatak and Panchal (2014)	Rat	50, 100 mg/kg BW (4% Tween 80)	p.o.	8 weeks	Improvement of the glycaemic status and renal function in diabetic rats with respect to marked normalization of the levels of creatinine, uric acid, blood urea nitrogen, albumin urinary
Kozuka et al. (2012)		20, 80, 320 mg/kg BW (0.5% methyl cellulose)	p.o. (by gavage)	13 weeks	Attenuation of preference for an high-fat diet by decreasing endoplasmic reticulum stress response in mice.
Wang et al. (2015)	Rat	0.16%	p.o. (*ad libitum*)	13 weeks	Improvement of serum lipid profile, ↑HDL. Increased adiponectin secretion. Decreased obesity and inflammatory markers levels. Hepatoprotective effect
Hagl et al. (2016)	Mice	340 mg/kg BW	p.o. (by gavage)	3 weeks	Neuro-protective effect, compensation of age-related mitochondrial dysfunction

BW, body weight; s.c., subcutaneous injection; i.v., intravenous injection; p.o., oral ingestion; HCD, high cholesterol diet; TC, total cholesterol; LDL, low-density lipoprotein; VLDL, very low-density protein; HDL, high-density lipoprotein; LPS, lipopolysaccharide, ADP, adenosine diphosphate.

cholesterol diet (HCD) (1% cholesterol 0.5% cholic acid), while groups III and IV were simultaneously supplemented by 0.5 and 2.0% GO respectively. The first group was administered a control diet. Total cholesterol (TC), very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) were improved by the administration of GO.

Likewise, rats receiving HCD (1% cholesterol) along with 100, 500 and 1000 mg/kg BW p.o. GO for 6 days had reduced serum TC levels compared with control animals (Nakayama et al., 1987). Surprisingly, this hypocholesterolaemic effect ceased after 12 days of supplementation (Nakayama et al., 1987). According to Rong et al. (1997), hamsters fed HCD (5% coconut oil 0.1% cholesterol) for 10 weeks with 0.5% or 1% GO for 7 weeks had decreased serum TC and non-HDL-C relative to control animals. More recently, Wilson et al. (2007) observed a similar effect in hamsters receiving lower doses of GO (0.5%) with HCD containing 5% more coconut oil (10% coconut oil, 0.1% cholesterol for 12 weeks, 0.5% GO for 10 weeks).

Some authors indicated poor intestinal absorption of vegetable sterols such as GO. Therefore, Sakamoto et al. (1987) showed a significant hypolipidaemic effect of intravenous administration of 10 mg/kg BW GO (daily for 6 and 12 days) in HCD rats. However, in the same study TC levels were not influenced by the oral administration of GO at 100 mg/kg BW (Sakamoto et al., 1987). In contrast to other studies, GO was also shown to have little or no preventive effect on hypercholesterolaemia in rabbits fed HCD (Hiramatsu et al., 1990).

High-density lipoprotein cholesterol, known colloquially as 'good cholesterol,' acts as a LDL scavenger. Thus, other authors (Suh et al., 2005; Wilson et al., 2007; Wang et al., 2015) postulated that HDL-increasing abilities contribute to GO's anti-atherosclerotic properties. More recently, Ghatak and Panchal (2012c) showed significant increases in HDL levels in WR1338-induced acute hyperlipidaemia in rats receiving GO in a dose of 100 mg/kg BW but not in those administered lower doses (50 mg/kg BW). Shinomiya et al. (1983) and Sakamoto et al. (1987) described a slight but not significant inhibition of the decrease in serum HDL. Contrasting results were presented by Nakayama et al. (1987) and Rong et al. (1997), who did not observe any effect of GO on HDL-C.

Another aspect of this anti-atherogenic action that was studied was the influence of GO on prevention of aortic fatty streak formation. Although evaluations of this action are inconsistent due to differing methodology, conclusions are similar: GO prevents aortic cholesterol accumulation. Shinomiya et al. (1983) used the ratio of LDL-cholesterol to acid cholesterol esterase and the ratio of acyl-coA cholesterol acyl-

Table 2 Gamma-oryzanol (GO) dosages used in *in vitro* experiments

Experiment	Cell type	Dose	Solvent	Duration of incubation	Efficiency/finding
Huang (2003)	Mouse lymph endothelial cells (SVEC4-10), human intestinal cells (C2BBel)	0.1–1 mmol/l	Synthetic micelles	1.0 mmol/1 h; up to 0.5 mmol/ 22 h for SVEC4-10; 0.1 mmol/ 2 h for C2BBe	No antioxidative effect after 1-h incubation, significant antioxidative effect after 22 h of pre-incubation; no GO uptake with intestinal cells was detected;
Ohara et al. (2009)	Mouse adipocytes	0.1; 0.5; 1; 5 μmol	DMSO	24 h	Tendency towards increased adiponectin secretion in physiological state and significantly increased adiponectin secretion under NFκB-activated state.
Mäkynen et al. (2012)	Human Caco-2 cells (HTB-37)	26 μmol/l	Synthetic micelles	4 h	Decreased cholesterol apical uptake
Sakai et al. (2012)	Bovine aortic endothelial cells, human umbilical vein endothelial cells	1–30 μmol/l	Not given	16 h	Reduced LPS-mediated adhesion molecule expression through NFκB inhibition
Kozuka et al. (2012)	Human embryonic kidney cells; murine primary neuron cells	10 μmol/l; 0.1 and 1 μmol/l	Not given	15, 24 h; 2 h	Decreased endoplasmic reticulum stress markers
Ismail et al. (2014)	Human neuroblastoma SH-SY5Y	1;10;100 μg/ml	Serum-free medium	24 h	Preservation of cell viability and morphology in the presence of H_2O_2. Expression changes of antioxidant (up), proapoptotic (down) and anti-apoptotic genes (up)
Wang et al. (2015)	Human hepatocellular carcinoma (HepG2)	50 μmol/l	Serum-free medium oleic acid-bovine serum albumin	24 h	Decreased triglycerides content, downregulation of hepatic lipogenesis-related genes

LPS, lipopolysaccharide.

transferase to neutral cholesterol esterase as markers of the deposition of lipid, especially cholesterol ester, in the aortic arch. Comparing GO-supplemented animals to control animals the first ratio was lower, while the other remained unchanged, suggesting that GO, at least in part, prevents cholesterol ester deposition. A few years later, Seetharamaiah et al. (1990) used a platelet aggregation model to study the influence of GO on prevention of aortic fatty streak formation. GO administered along with HCD significantly inhibited platelet aggregation induced by adenosine diphosphate and totally inhibited aggregation induced by collagen. This was not observed with the control diet. Likewise, aortic fatty streak formation, defined by the degree of accumulation of oil red O-stained macrophage-derived foam cells, was reduced 67% ($p < 0.01$) in the GO-treated animals (Rong et al., 1997). The authors postulated that this was associated with the reduction in plasma non-HDL-C concentrations. Decreased amounts of cholesterol accumulation in the aortic arch of hamsters were confirmed in a subsequent study performed at the same laboratory (Wilson et al., 2007). A recent *in vitro* study suggests a possible molecular mechanism of GO inhibition of endothelial adhesion (Sakai et al., 2012). Pre-treatment with a GO dose correspondingly decreased lipopolysaccharide (LPS)-induced expression of nuclear factor-$\kappa\beta$ (NF$\kappa\beta$) and adhesion molecules: vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and E selectin expression in bovine aortic endothelial cells, as well as VCAM-1 expression in human umbilical vein endothelial cells.

It is now widely acknowledged that the hypocholesterolaemic effect of plant sterols is mainly due to their ability to inhibit cholesterol absorption from the intes-

tine. Shinomiya et al. (1983) were the first to adapt this thesis for GO. Numerous studies confirmed this thesis (Nakayama et al., 1987; Rong et al., 1997; Wislon et al., 2007), while Seetharamaiah and Chandrasekhara (1990) investigated it in detail and distinguished two mechanisms of the prohypocholesterolaemic action of GO: inhibition of cholesterol absorption and increased faecal excretion of bile acids. Feeding rats a GO diet vs. a control diet did not bring about any change in bile flow and composition, but nearly doubled cholesterol faecal excretion. In a group fed GO along with an HCD diet, bile flow and bile acid output were increased 12% and 18% respectively, while biliary cholesterol remained unchanged. The increased bile acid secretion was mainly due to taurocholic acid. There was a significant increase in the faecal excretion of cholesterol (28%) and bile acids (29%), whereas cholesterol absorption was lowered by 20%. According to Mäkynen et al. (2012), other possibilities for the hypocholesterolaemic activity of GO include interference with the incorporation of cholesterol into micelles during small intestinal digestion. Although incorporation of cholesterol into synthetic micelles was significantly inhibited by 1500 μmol GO (15 fold molar excess compared to cholesterol), the efficiency of micellarization of cholesterol during simulated digestion of rice meal was not significantly altered by the presence of as high as 2000 μmol GO (20 fold molar excess compared to cholesterol). Nevertheless, GO significantly decreased apical uptake of 14C-cholesterol delivered in mixed micelles into Caco-2 human intestinal cells.

Contrasting results are given by Huang (2003), who did not demonstrate any significant effect of 24-h incubation of GO on micellar solubility of cholesterol and cholesteryl oleate, although the molar ratio of cholesterol to GO is not given by the author. In addition, this study did not confirm the GO effect on cholesterol apical uptake; however, molar ratios of experimental factors were 1:1 and the *in vitro* model used large intestine cells, while absorption of cholesterol takes place in small intestine. The author also observed that GO has a tendency toward the inhibition of cholesterol esterase, the enzyme responsible for hydrolysis of cholesterol esters in the intestine. This could be in agreement with the previous notion showed in digestibility section that some compounds of GO are susceptible to CE and therefore may compete with cholesterol during digestion process.

A review of several studies also indicated that GO affects the activity of HMG CoA reductase–the rate-limiting enzyme of cholesterol synthesis (Mäkynen et al., 2012), but the presented results are equivocal and require further investigation (Rong et al., 1997; Chen and Cheng, 2006).

The majority of research mentioned in this chapter does not provide information concerning the detailed amount of GO consumed by individual animals, because GO doses are expressed in percentage values. This may raise doubts about its credibility. Nevertheless, the antihyperlipidaemic action of GO seems to be indisputable and to occur primarily by reducing cholesterol absorption.

Gamma-oryzanol – a powerful antioxidant

Oxidative stress is caused by the generation of reactive oxygen species (ROS) under physiological and pathological conditions. It has been suggested that ROS are involved in numerous metabolic disorders such as cardiovascular diseases, cancer, cataract and age-related macular degeneration, neuronal diseases and so on. Xu and Godber (2001) suggested that the nutritional function of GO components may be related to their antioxidant functions due to the ferulic acid structure.

The antioxidant activity of GO has been evaluated in a few chemical models. One of these is a cholesterol oxidation model accelerated by 2,2′-azobis(2 methylpropionamidine) dihydrochloride (AAPH), which was established to compare the inhibitory capability of Vitamin E (alpha-tocopherol, alpha-tocotrienol, gamma-tocopherol and gamma-tocotrienol) and GO components (cycloartenyl ferulate, 24-methylenecycloartenyl ferulate and campesteryl ferulate) purified from rice bran on the production of oxidized cholesterol (Xu et al., 2001). The results suggested that 24-methylene cycloartenyl ferulate had the highest antioxidant activity and all three major components of GO had higher antioxidant activity than vitamin E components.

The antioxidative activity of the three major components of GO was also addressed in a linoleic acid model. Antioxidative activity was determined to be the degree of inhibition on hydroperoxide formation that resulted from linoleic acid incubation with air. The three components of GO evidenced significant antioxidative activity when they were mixed with linoleic acid in a molar ratio of 1:100 and 1:250 but not in a molar ratio of 1:500. Tocopherol and ferulic acid also demonstrated significant antioxidant activity at all three molar ratios (Xu and Godber, 2001).

The *in vitro* model employing a mouse lymph axillary endothelial cell line SVEC4-10 was presented by Huang (2003). The author evaluated antioxidant features of various concentrations of GO, ferulic acid and tocopherol, and possible synergism among them, in

the *tert*-butyl hydroperoxide (tBHP) oxidative stress model. Three major components of GO were also investigated: cycloartenyl ferulate, 24-methylene cycloartenyl ferulate and campesteryl ferulate. Simultaneous incubation of experimental factors with tBHP for 1 h showed no significant oxidative damage-preventing effect on examined cells. On the other hand, 22 h pre-incubation with experimental factors showed a significant antioxidative effect. The three major components of GO investigated separately had higher antioxidant activity than GO. Among them, 24-methylene cycloartenyl ferulate was found to be relatively more effective. A synergistic antioxidant activity of GO, ferulic acid and α-tocopherol was also found when these compounds were simultaneously incubated with tBHP for 1 h. Gamma-oryzanol protected cells from tBHP oxidative damage in a time-dependent manner; therefore, the author suggests that pre-incubation allowed binding with the cell membrane, which was required for antioxidant protective activity.

Since there are multiple ways in which a substance can exert its antioxidant activity, Juliano et al. (2005) decided to assess the antioxidant mechanism of gamma-oryzanol with different *in vitro* models. They applied two experimental models: one including inorganic oxygen-derived radicals, such as OH• and O2•− and organic radicals: 2,2-azobis(2,4-dimethylvaleronitrile) and the lipid-soluble 2,2-Diphenyl-l-picrylhydrazyl (DPPH•) radical. The authors did not prove that GO can be an OH• or O2•− scavenger. However, GO was able to inhibit lipid soluble organic radicals when incorporated within liposomes. This is in agreement with results by Xu et al. (2001) and Huang (2003).

Few *in vivo* trials addressed GO's antioxidative potential. The first, Accinni et al. (2006) showed the advantages of a 4-month combined dietary supplementation with PUFA n-3, vitamin E, niacin and GO on oxidative stress levels in dyslipidaemic patients. Among three experimental groups, the one consuming GO had the best oxidative stress rates expressed by: ROS, interleukin 1-b (IL1-b), tumour necrosis factor (TNF-a) and thromboxane B2 (TXB2) concentration and total antioxidant capacity (TAC).

Chotimarkon and Ushio (2008) observed the antioxidant effects of a 30-day oral administration of 0.02 mmol GO per animal (680 mg/kg BW) in reversing ethanol-induced liver injury in the C57BL mouse. It was demonstrated by markedly decreased serum plasma aspartate aminotransferase, alanine aminotransferase and significant decreases in hepatic lipid hydroperoxide and thiobarbituric acid reactive substance levels in GO vs. the control condition. Furthermore, GO-treated mice better recovered from an ethanol-induced decrease in the hepatic glutathione level together with enhancing superoxide dismutase activity. GO effectiveness in ameliorating streptozotocin-induced oxidative stress in rats (Ghatak and Panchal, 2012b) is detailed in the chapter concerning diabetes management.

In addition, thoroughbred horses receiving GO during a 16-week training season had significantly lower post-exercise total antioxidant status and thiobarbituric acid reactive substance level (Ostaszewski et al., 2012).

Recently considerable attention has been devoted to the molecular background of antioxidant properties of GO. Ismail et al. (2010) determined the antioxidant properties of GO on rats' liver gene expression changes. The animals were subjected to a 10-week swimming exercise programme in order to induce oxidative stress. For the last 5 weeks of this programme, GO was administered. The authors suggest that downregulation of ubiquitin B (Ubb), stress-induced phosphoprotein 1 (Stip1), NFκβ and oxidative stress responsive 1 (Oxsr1) genes and upregulation of hydroxyacid oxidase 1, liver (Hao1), apolipoprotein E (ApoE), mettalothionein (Mt1), superoxide dismutase (SOD) and catalase (CAT) mRNA levels proves GO to be a potent antioxidant. Similar expression regulation was noted in human neuroblastoma cells exposed to hydrogen peroxide (Ismail et al., 2014).

Concluding, *in vivo* trials proved GO to be powerful antioxidant, while *in vitro* results suggest that GO is able to inhibit the formation of new free radicals as well as scavenge lipid soluble organic radicals. While it is true that the above-mentioned results agree with the hypothesis of GO antioxidant activity, the efficacy of GO against inorganic oxygen-derived radicals still remains unclear.

Gamma-oryzanol – promising anticancerogenic compound?

Crude RBO and numerous RBO ingredients were investigated in terms of their anticancerogenic action. Fermented brown rice and rice bran prevented colorectal carcinogenesis in mice (Phutthaphadoong et al., 2010) and rats (Katyama et al., 2002), lung tumorigenesis (Phutthaphadoong et al., 2009) in mice, hepatocarcinogenesis (Katyama et al., 2003), and oral carcinogenesis (Long et al., 2007) in rats. Ferulic acid from brown rice inhibited growth of human breast and colon cancer cells (Hudson et al., 2000). Phytic acid from rice bran induced apoptosis in

human colorectal adenocarcinoma cells (Shafie et al., 2010) and inhibited colon carcinogenesis in rats (Norazalina et al., 2010). Tocotrienol-rich fraction isolated from rice bran oil inhibited diethylnitrosamine and 2-acetylaminofluorene-induced hepatocarcinogenesis in rats (Iqbal et al., 2004). Furthermore, the main ingredient of GO: cycloartenol ferulate, markedly inhibited the tumour-promoting effect of O-tetradecanoylphorbol-13-acetate (Yasukawa et al., 1998). The impact of RBO and its derivatives appears to be well documented; however, studies examining GO's anticancer properties are scarce and presented results are ambiguous.

Kim et al. (2012) investigated the effects of rice bran and its components on tumour growth in mice. Mice were fed standard diets enriched with rice bran, GO, Ricetrienol R, ferulic acid or phytic acid for 2 weeks. Then animals were inoculated with CT-26 colon cancer cells and continued the same diet for two additional weeks. The mass of the growing tumour was significantly lower in the GO and less so in the phytic acid group. Tumour inhibition was associated with the following biomarkers: increased cytolytic activity of splenic natural killer cells (NK); partial restoration of nitric oxide production and phagocytosis in peritoneal macrophages; increased levels of pro-inflammatory cytokines released from macrophages (tumour necrosis factor-α, IL-1β, and IL-6); and reduced number of blood vessels inside the tumour. mRNA and protein levels of proangiogenic biomarkers: vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2) and 5-lipoxygenase-5 (5-LOX), were also significantly reduced inside the tumours. The authors postulated that this may indicate inhibition of neoangiogenesis inside the tumours. Three concentrations of GO were examined 0.2%; 0.5%; and 1%, served *ad libitum*; however, the authors did not provide any data about food consumption; thus, it is difficult to conclude what the exact dose of the supplement was. Nonetheless, all dosages proved to be beneficial, but anticancerogenic effects were the most pronounced in the highest concentration.

In contrast to the above-mentioned report, Hirose et al. (1998) showed that GO may increase the incidence of multiplicity of lung tumours in a multi-organ carcinogenesis model in male F344 rats. During the first 3 weeks, the animals received combined treatment consisting of 3 carcinogens: 2,2′-dihydroxy-di-n-propylnitrosamine (DHPN), N-ethyl-N-hydroxyethyl-nitrosamine (EHEN) and 3,2′-dimethyl-4-aminobiphenyl (DMAB), and then were treated with 1% gamma-oryzanol or basal diet for 32 weeks. Carcinogenesis-enhancing effects of GO on lungs were observed at a dose of 1% but not at 0.5% or lower. Similarly, enhancement of lung carcinogenesis by 1% GO was reported by the same author in 1991. The second part of the study examined the potential of phytic acid and GO on mammary carcinogenesis in rats. GO-pre-treated animals revealed no significant differences in the final incidences and multiplicities of mammary tumours. GO tended to decrease the size of tumours but without significant difference.

It is widely acknowledged that the NF$\kappa\beta$ signalling pathway has several implications in cancer**.** Constitutive activation of NF$\kappa\beta$ has been observed in different forms of cancer, including lymphoma, leukaemia, breast, colon, liver, pancreas, prostate and ovarian cancers. Moreover, activation of NF$\kappa\beta$ has been linked to recurrence, poor survival rates, tumour progression, aggressiveness and chemoresistance (Jing and Lee, 2014). There is plentiful research evidence that GO anti-inflammatory and antioxidative activity is mediated by inhibition of NF$\kappa\beta$ activity (Islam et al., 2008; Ismail et al., 2010; Sakai et al., 2012). Thus, the anticancerogenic action of GO might to some degree be mediated by NF$\kappa\beta$ pathway regulation.

In summary, according to Kim et al. (2012), GO appears to be a very promising anticancerogenic compound. Moreover, as mentioned above, biosafety studies performed by Tamagawa et al. (1992a,b) showed that the tumour incidents were not significantly different between the treated and control groups after feeding high doses of GO to mice and rats for up to two years. The most significant difference between Hirose et al. (1998) and Kim et al. (2012) is inverse order of GO treatment and carcinogen application. In the first study, GO was administered after introduction of carcinogen while in the other animals were pre-treated with GO for 2 weeks. It is difficult to explain such contrasting results of both authors, although it may be suggested that GO is beneficial in prevention rather than in treatment of cancer. Further, tumour cells in each study originated from a different tissue. Additional research is required on this topic.

Gamma-oryzanol in enhancing muscle strength – fact or myth?

Gamma-oryzanol and its related compound ferulic acid are believed to have antioxidant properties that affect hormone function in the body, resulting in anabolic effects on muscle growth as well as reduced fatigue. Due to its touted properties, many companies offer feed supplements for horses that contain GO as a naturally occurring anabolic substance. Although so far its effectiveness remains ambiguous, the Federa-

tion Equestre Internationale unit governing rules of fair play in equestrian competition, listed GO as a prohibited substance (medication class B) (https://www.usef.org/documents/drugsMeds/FEIVetRegs.pdf). Thus, manufacturers often warn that the use of GO products may contravene the rules of equestrian competition, and recommend withdrawal time before competition. Because GO is insoluble in water, a GO emulsion is used in supplementing humans, dogs and horses. Despite its apparent use as an ergogenic aid, only a few studies of ergogenic use of GO were described in the peer-reviewed literature.

Gamma-oryzanol is postulated to lessen muscle exhaustion and fatigue in response to anaerobic exercise, and to enhance muscle building capacity by hindering the production of free radicals in muscle tissue (Eslami et al., 2014). However, to date studies have failed to demonstrate any beneficial effects of using antioxidants in human (Bernstein et al., 2002) and animal (Piercy et al., 2000; White et al., 2001) athletes. Ostaszewski et al. (2012) was the first who showed that supplementation of powerful antioxidant – GO – helps to prevent exercise-induced muscle damage in thoroughbred horses during 16 weeks of training in preparation for the racing season. The animals received 3 g of GO daily. Analysis of variance showed a significantly greater increase in post-exercise creatinine kinase and blood lactate activity in the placebo-supplemented group than in the GO-treated groups, both in the training period and during the racing seasons. Although the exact mechanism of the compound's activity was not revealed, horses receiving GO had significantly lower post-exercise total antioxidant status and thiobarbituric acid reactive substance level than horses from other groups. Whether GO antioxidant activity in muscle tissue exceeds other naturally occurring antioxidants remains to be clarified. Another question that should be answered is whether post-exercise ROS production differs in various species and how the duration of GO supplementation may influence its efficacy.

Regardless, GO is consumed in the belief that it may elicit anabolic effects ranging from increased testosterone production to enhanced human growth hormone release. However, animal studies indicated, that when administered parenterally, GO induces anti-anabolic or catabolic activity. Intravenous or subcutaneous injections of GO in rats have been shown to suppress LH release, reduce GH synthesis and release and increase release of the catecholamines, dopamine and norepinephrine, in the brain. Although it has still not been directly measured, this metabolic milieu may actually reduce testosterone production (Cicero and Gaddi, 2001). Mösseler et al. (2010) investigated whether oral intake of GO can affect urine and serum testosterone levels in healthy endurance horses. Six horses (three mares, three geldings) received GO in a dose 2 g/500 kg BW daily for 31 days. The horses were trained daily. No influence of GO on serum and urine testosterone was demonstrated; however, the small number of tested animals raises doubts about the reliability of the statistical survey.

Few GO-related human studies exist and those that do seem to raise more questions than to provide answers. Fry et al. (1997) carried out a study among 22 weight-trained adults, randomly divided to consume either 500 mg of GO or a placebo, each day throughout a nine-week periodized strength exercise programme. Body composition, muscle strength, power, heart rate, blood pressure and levels of circulating hormones (testosterone, cortisol, oestradiol, GH, insulin, beta-endorphin) were compared, and in spite of the fact that performance measures were enhanced, no differences were noted among the GO and placebo groups, which showed that ingesting GO throughout a strength training programme had no effect. On the contrary, Bucci et al. (1990) showed that only 30 mg per day of ferulic acid ingested for 8 weeks in trained weight lifters increased body weight and strength. However, the percentage of lean body mass in weight gain was not determined. Strength was measured by one maximum lift and only 10 athletes took part in the measurement. In a recent study, Eslami et al. (2014) revealed that 600 mg/day GO supplementation changed muscular strength in young healthy males in a 9-week resistance training regimen without any significant alteration in anthropometric measurements. During the first phase of strength training, most of the strength gain arise from neural adaptations; therefore, as suggested by the authors, the absence of alterations in body changes and muscle enhancements could be explained by the fact that untrained individuals were selected.

In conclusion, up to date studies show that long-term consumption of GO may prevent post-exercise muscle damage in horses, due to its capacity to improve the antioxidative profile. However, further studies on the effects of GO on lean body mass both in athletes or exercised animals should be conducted to validate the use of this ergogenic supplement.

Gamma-oryzanol in diabetes management

Due to the absence of effective and affordable interventions, prevalence and treatment of diabetes has gained global interest during the last decade.

A study investigating the effect of GO-enriched experimental diet, on the blood glucose level in diabetic mice, showed a lower concentration of fasting blood glucose and blood glucose area from a glucose tolerance test as compared to the control group. Serum insulin level was higher in the experimental group but without statistical significance (Lee et al., 2004). In contrast, Frank et al. (2005) showed that, a four-week consumption of 240 ml/daily of corn oil, refined RBO or cRBO lowered insulin sensitivity, but combined intravenous glucose-insulin tolerance tests, plasma glucose and insulin concentrations remained unaffected by the oil supplementation. However, published data include only averaged values for all three oils while only cRBO is rich in the unsaponifiable fraction containing GO (Patel and Naik, 2004).

A high-fat diet, besides its unquestionable role in pathogenesis of obesity and cardiovascular disease, is one of the main risk factors in type 2 diabetes mellitus as well. Due to this, the antihyperlipidaemic abilities of GO may be essential in diabetes management. It was confirmed recently by Wang et al. (2015) who demonstrated GO efficacy in alleviating the high-fat, high-fructose-induced metabolic syndrome. Obesity, hyperlipidaemia, hyperglycaemia, hepatic injury, hepatic lipid accumulation, insulin resistance and decreased inflammatory marker levels were improved in rats receiving 0.16% of pure GO for 13 weeks vs. the control. Moreover, regulation of lipogenesis-related gene expression by GO was demonstrated *in vitro*. Similar improvement of high-fat diet-induced glucose dysmetabolism was observed in two other studies (Son et al., 2011; Kozuka et al., 2012).

In addition to its antihyperlipidaemic action, several different mechanisms have been implicated in GO's action against diabetes; however, GO's limited digestibility and poor absorption may question these effects. Ohara et al. (2009) showed that GO might be effective for ameliorating type 2 diabetes through regulation of adiponectin secretion. In mice, serum adiponectin concentrations were increased by oral administration of 3.125 and 12.5 μmol GO, but not by 25 μmol. Recently, Wang et al. (2015) presented similar results. A tendency towards decreased adiponectin secretion was observed *in vitro* after 24-h exposition of mice adipocytes to 0.5 and 1.0 μmol GO; however, statistical significance was reached only in cells under the NF$\kappa\beta$ activation state. The authors concluded that GO might regulate adiponectin secretion by inhibiting NF$\kappa\beta$ activation. This finding is in agreement with other studies (Sakai et al., 2012). Moreover, nutritive compounds may control adipocytes secretion *in vivo* through a variety of hormones, growth factors and metabolites, but there is little evidence to suggest that GO may directly interact with cells *in vitro*.

One of the mechanisms underlying diabetes and its related complications is increased oxidative stress (Moussa, 2008). In diabetes, increased production of free radicals, especially ROS, causes persistent hyperglycaemia and can initiate peroxidation of lipids, which in turn stimulates non-enzymatic glycation of protein, inactivation of enzymes and alterations in the structure and function of collagen, which collectively produces the late diabetic complications (Baynes, 1991). GO antioxidant properties were investigated in the *in vivo* model of streptozotocin (STZ)-induced diabetes (Ghatak and Panchal, 2012b). STZ is able to cause selective oxidative damage of pancreatic β-cells. A significant decrease in lipid peroxidation and glutathione in the liver, as well as an increased level of the enzymatic antioxidant: superoxide dismutase indicates that GO effectively ameliorates STZ-induced oxidative stress. Furthermore, administration of GO dose dependently decreased glucose levels in normoglycaemic and hyperglycaemic rats, which was attributed to the changes in GK, PEPCK and G6pase activities similar to those reported by Son et al. (2011). Subsequent study by the same author showed that 8-week-long GO supplementation proffered marked protection against diabetic nephropathy with respect to the normalization of the levels of creatinine, uric acid, blood urea nitrogen, albumin, urinary albumin to creatinine ratio, kidney tissue enzyme, total protein, serum lipid profile and electrolyte concentration in a dose-dependent manner. Histopathological observations also evidenced regression in renal pathological alterations in GO-treated animals (Ghatak and Panchal, 2014).

In conclusion, supplementation of GO in diabetic patients may improve metabolic dysfunctions such as hyperglycaemia, hypercholesterolaemia, hypertriglyceridaemia, insulin resistance and may alleviate diabetic complications resulting from lipid peroxidation and free radicals.

Other effects of gamma-oryzanol

One of the properties of GO that was extensively studied in the 1970s in different animal models was its anti-ulcerogenic action (Cicero and Gaddi, 2001).

The preventive action of GO has been demonstrated in various types of experimental ulcers: water immersion stress ulcers (Itaya and Kiyonaga, 1976), conditioned emotional stimuli (communication box method) and rapid eye movement (REM) sleep deprivation ulcers (Ichimaru et al., 1984), vagal

stimulants-insulin or 2-deoxy-D-glucose-induced ulcers (Mizuta and Itaya, 1978). Moreover, GO administration inhibited tetragastrin-stimulated gastrin secretion (Mizuta et al., 1978) which contrasts to the slight effects on ulcers induced by pylorus-ligation, stress-atropine as well as histamine- and carbachol-stimulated acid secretion (Mizuta et al., 1978).

Multiple mechanisms of anti-ulcerogenic action were proposed for GO. Itaya and Kiyonaga (1976) suggested the autonomic nervous system, but not the hypophysis–adrenal axis participation in the anti-ulcer action of GO due to the positive response of adrenalectomized as well as sham-operated ulcerogenic rats. Inhibitors or precursors of catecholamines were shown to modify the anti-ulcerogenic action of GO (Itaya et al., 1976). Treatment with reserpine prior to stress loading eliminated the anti-ulcer effect of GO given subcutaneously for 5 days. However, the administration of L-DOPA or 5-hydroxytryptophan

Fig. 3 A summary of gamma-oryzanol (GO) mechanisms of action. Abbreviations: endoplasmic reticulum (ER), low density lipoprotein (LDL), high-density lipoprotein (HDL), reactive oxygen species (ROS). Increases (↑) or decreases (↓) in activity were indicated.

revealed a tendency toward restoration of the anti-ulcer effect of GO. The conclusion of these observations was that the monoaminergic neurone system is involved in GO anti-ulcer action. Moreover, it is assumed that the gastric antisecretory effect of GO is mediated by the vagus nerve, which plays a role in the action of gastrin (Mizuta et al., 1978). This hypothesis is confirmed by Itaya et al. (1977) who demonstrated a reduction in gastrin release induced by GO administration in rats. Likewise, pre-treatment with 100 mg/kg BW GO s.c. for 5 days depressed gastric secretion stimulated both by vagal stimulants: insulin and 2 deoxy-D-glucose, but the potency was less than that with 10 mg/kg BW s.c. atropine (Mizuta and Itaya, 1978). In addition, modulatory effect on gastrointestinal motility was observed in dogs (Mizonishi and Semba, 1980) and the facilitation of small intestinal propulsion induced by convulsive electroshock in responder-mice was suppressed by GO at 100 and 200 mg/kg BW and atropine at 10 mg/kg BW (Ichimaru et al., 1984).

The findings from the experimental animal models also demonstrate the immunostimulatory potential of GO. Cell-mediated immunity was assessed by the augmented phagocytic index of reticulo-endothelium system cells, the increase in delayed-type hypersensitivity reaction expressed by the enhanced thickness of the footpad after injection of sheep erythrocytes in rats who were administered 100 mg/kg BW GO for 21 days. Also, cyclophosphamide-induced myelosuppression was ameliorated as shown by elevated body weight, thymus and spleen mass, and by improved haematological parameters in animals supplemented with GO. Gamma-oryzanol also stimulates humoral immunity as indicated by an increased haemagglutinating antibody titre in rats. Thus, from the results obtained, it can be concluded that GO has therapeutic potential and could serve as an effective immunomodulatory candidate. However, the results from this study do not provide enough information to make recommendations for GO. Therefore, further studies are necessary in order to examine the therapeutic effectiveness of GO in conditions of immune depression, in the prevention of autoimmune diseases and its detrimental effects on inflammatory conditions (Ghatak and Panchal, 2012a).

Recent studies investigated the neuro-protective properties of GO. According to Ismail et al. (2014), GO can prevent oxidative stress-induced neurotoxicity *in vitro*, which was accompanied by protection of mitochondrial metabolic enzyme activities, and reduced apoptosis. The mechanistic basis for the neuro-protective effects of GO included up-regulation of antioxidant genes, downregulation of pro-apoptotic genes and upregulation of anti-apoptotic genes. Another study showed that 13 weeks of GO supplementation (320 mg/kg BW) significantly suppressed the expression of endoplasmic reticulum (ER) stress responsive genes in brain tissue, and reduced preference for an high-fat-diet in mice. Endoplasmic reticulum stress provokes leptin resistance in the hypothalamus, which leads to hyperphagia and obesity. This finding was reinforced by the data that GO reduced significantly tunicamycin-induced ER stress in murine primary neuronal cells (Kozuka et al., 2012). Hagl et al. (2016) showed that 3 weeks of supplementation of 340 mg/kg BW GO-rich rice bran extract compensated age-related mitochondrial dysfunction in brains of 18-month-old mice. This was manifested by increased mitochondrial respiration, membrane potential, peroxisome proliferator-activated receptor gamma coactivator 1-alpha protein expression and citrate synthase activity. Furthermore, resistance of brain cells to sodium nitroprusside-induced nitrosative stress was improved. This suggests that GO may be promising candidate for the prevention of age-related neurodegenerative diseases such as Alzheimer's disease.

Conclusion

Gamma-oryzanol appears to be safe and effective in ameliorating metabolic syndrome accompanying many pathological conditions including obesity, diabetes and cardiovascular disease. Recent studies suggest beneficial effects of GO in age-related neurodegenerative disease prevention. A summary of GO properties and possible mechanisms of action is shown on Fig. 3. Seen from the standpoint of the growing incidence of cancer, GO seems to be a very promising compound. However, more research is needed to confirm these data.

It can be concluded that the majority of beneficial effects of GO are due to its antihyperlipidaemic and antioxidative properties. Nevertheless, the exact mechanism of this compound's activity remains to be discovered. Results obtained in animal models should be supported by human studies.

Given the number of GO's reported properties, and its accessibility for commercial use, it is difficult to understand the lack of reliable digestibility and pharmacokinetics research. Doses, the type of solvent used in trials, and implications of the solvent type on intestinal absorption should be standardized to allow in-depth analysis of the results.

Another contradictory aspect of GO supplementation is its apparent use as an ergogenic aid. So far, only two studies: one in race horses and one in weightlifters, have supported anecdotal data showing GO's muscle enhancing properties. This offers new opportunities for studies on GO supplementation in racing and endurance animals.

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