Gamma-Oryzanol – A Multi-Purpose Steryl Ferulate

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Abstract: For the last couple of decades, there has been a surge of global interest pertaining to the beneficial nutritive effects of bioactive phytochemicals like γ- oryzanol (γ-OZ), obtained from crude rice bran oil (RBO), which is manufactured from rice bran, a by-product of rice processing. Oryzanol, although presumed to be a single component initially, was shown to be a mixture of ferulic acid esters of triterpene alcohols and plant sterols. The γ-OZ component can be simultaneously separated and quantified by high performance liquid chromatography, once the RBO has been extracted from rice bran by solvent extraction using food grade n-hexane or by supercritical fluid extraction technology. A number of potentially therapeutically useful biological activities have been reported for γ-OZ, in terms of improvement of the plasma lipid pattern of rodents, rabbits, non-human primates and humans, reducing total plasma cholesterol and triglyceride concentration and cholesterol absorption from cholesterol-enriched diets and aortic fatty streaks and simultaneously increasing the high density lipoprotein cholesterol level. Other potential properties of γ- OZ, that have been studied both *in vitro* as well as *in vivo* are the modulation of the pituitary secretion, inhibition of the gastric acid secretion, antioxidant action and inhibition of the platelet aggregation. However, these studies were unable to produce unequivocal conclusions and had been conducted on animal species very different from each other, and using diverse experimental methodologies and targets. The current contribution provides a comprehensive review of the chemical constituents, pharmacological profile and the healthcare properties of γ-OZ as a nutraceutical.

Keywords: Anti-oxidant, extraction, γ-oryzanol, *in vivo*, prevention, rice bran oil.

INTRODUCTION

In recent times, there has been an emerging interest in the use of naturally occurring phytochemicals for their potential therapeutic usage in minimizing the risk of major chronic diseases like cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts, and age-related functional decline [1-3]. In the light of the same, rice bran obtained during milling of rice (*Oryza sativa*) is gaining surmount commercial importance in the world on account of its innumerable beneficial nutritive and biological effects with 16 –22% lipid, 12 –16 % protein, 8-12 % crude fiber and high levels of other vitamins and minerals [4]. As a result of developments in the stabilization of rice bran and the increase in knowledge about health benefits associated with rice bran oil (RBO), extraction of RBO by solvent extraction using food grade n-hexane or in solvent free process by using ohmic heating or supercritical fluid extraction technology has received greater attention [5-7].

In contrast to most common refined vegetable oils, crude RBO contains a rich unsaponifiable fraction (up to 5%) mainly constituting of sterols (43%), triterpene alcohols (28%) 4-methyl-sterols (10%) and less polar components (19%) [8]. Phytosterols include β-sitosterol (900 mg%), campesterol (500 mg%), stigmasterol (250 mg%), squalene (320 mg%) and γ- OZ (1.6%). The so-called γ- OZ often identified as the physiologically active moiety of rice bran oil, is a mixture of ferulate (4-hydroxo-3- methoxycinnamic acid) esters of sterols (campesterol, stigmasterol and β-stigmasterol) and triterpene alcohols (cycloartenol, cyccloartenol, 24-methylenecycloartanol, cyclobranol). Major portions of γ- OZ include cycloartenyl ferulate, 24 –methylene cycloartanyl ferulate and campesteryl ferulate (Fig. **I** (**a**), **I** (**b**) and **I** (**c**)). γ- OZ is 1.5 –2.9 % of rice bran oil and is white or yellowish odorless, tasteless powder with a melting point of 137.5~138° C [9, 10].

The quantitative determination of γ- OZ is very difficult to achieve because it is a mixture of many ferulic esters, and a number of determination methods have been proposed for the same including absorptiometry and high performance liquid chromatography [11, 12].

The current review extensively focuses on the extraction of γ- OZ from RBO, accompanied by its pharmacokinetics, its potentially therapeutically useful biological activities and diverse healthcare properties.

EXTRACTION AND PURIFICATION OF Γ-OZ

Extraction and purification of OZ from RBO have been extensively reviewed. For extraction-based processes, critical process parameters that need to be evaluated are solid to solvent ratio, temperature, and time [13]. Organic solvent extraction, although is the conventional method for extraction of γ-OZ, it uses highly toxic and highly flammable solvents and has problems of waste disposal and leaving toxic residues. Hence, the search for alternative, non-hazardous and environment-friendly extraction technique has lead to the

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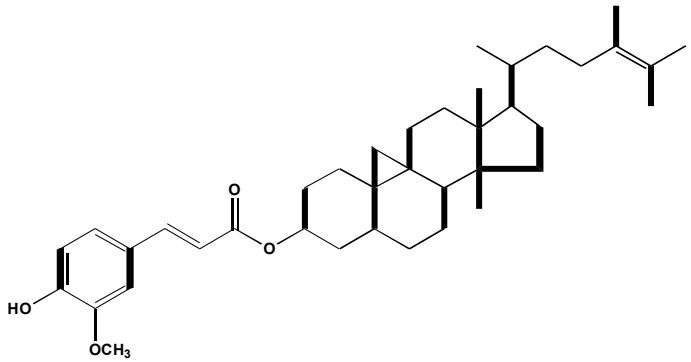

Fig. I(a). Cycloartenyl ferulate.

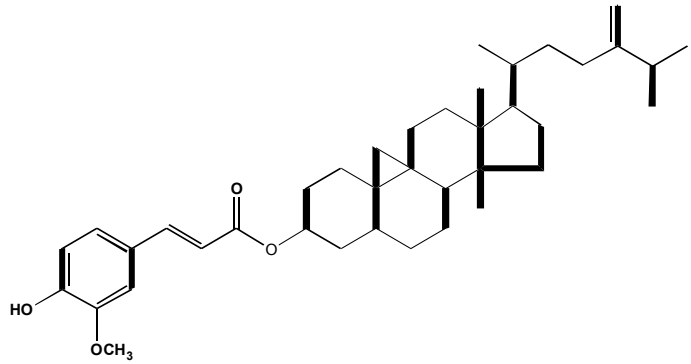

Fig. I(b). 24 –methylene Cycloartanyl ferulate.

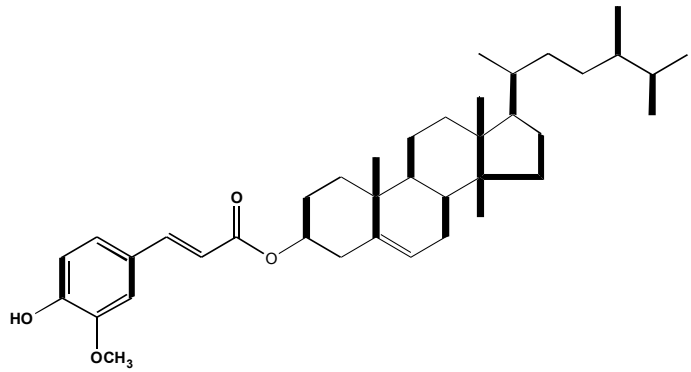

Fig. I(c). Campesteryl ferulate.

emergence of supercritical fluid techniques for the extraction procedures. Xu and Godber have compared the supercritical fluid and solvent extraction method for extracting γ-OZ from rice bran [14]. They found that a solvent mixture with 50% hexane and 50% isopropanol (vol/vol) at a temperature of 60°C for 45–60 min produced the highest yield (1.68 mg/g of rice bran) of γ-OZ. However, the yield (5.39 mg/g of rice bran) of γ-OZ in supercritical fluid extraction under a temperature of 50°C, pressure of 68,901 kPa (680 atm), and time of 25 min was approximately four times higher than the highest yield of solvent extraction. Moreover, a high concentration of γ-OZ in the extract (50–80%) was obtained by collecting the extract after 15–20 min of extraction under optimized conditions.

Published reports cited in the literature for the isolation of γ-OZ from RBO using preparative HPLC, silica-based continuous chromatography combined with multistage of crystallization exposed some serious limitations of the existing techniques, like low productivity, use of chlorinated or aromatic toxic solvents like benzene, multi-stage processes and methods not reproducible to production scale [15-17]. To overcome these limitations, Zullaikah *et al* have recently reported a two-step crystallization process for the isolation of γ- OZ from crude RBO [18]. In the first crystallization step, γ- OZ was concentrated in the liquid phase along with free fatty acid (FFA), monoacylglycerol (MG), squalene, tocols, and phytosterols, whereas the solid phase contained mainly triacylglycerol (TG) and steryl esters. OZ-rich product obtained from the first crystallization was subjected to the second crystallization where the OZ-rich product was kept at room temperature (20.5 ± 1.5°C) for 24 h. Hexane was added as an anti-solvent to the OZ-rich product and kept at 5 ± 1°C for another 48 h, after which white OZ crystals with a purity and recovery of 93-95% and 59%, respectively were obtained.

BIOLOGICAL ACTIVITY

In vitro Antioxidant and Free Radical Scavenging Activity

At the cellular and molecular levels, antioxidants play a prominent role in deactivating the free radicals that can cause damage to cell walls, certain cell structures and genetic material within the cells. Identification of oxidant and antioxidant bioactive compounds is important, not only for predicting and reducing health related risks, but also for evaluating possible combinatory beneficial effects of bioactive ingredients in phyto-therapeutic formulations. One test-tube study found that at $10^{(-4)}$ M concentration, γ-OZ was more than four times as effective at stopping tissue oxidation as vitamin E, the most effective antioxidant due to its abundance in the body [19]. The nutritional function of γ-OZ components may be related to their antioxidant property on account of its fundamental molecular structure i.e. the ferulic acid aromatic phenolic nucleus esterified to cyclopentanperihydrophenanthrene [20].

However the antioxidant capacities of γ-OZ components were not explored until Xu *et al.* (2001) evidenced significant antioxidant activity for the three major components of γ-OZ (24- methylene cycloartanyl ferulate, cycloartenyl ferulate and campesteryl ferulate) in a linolenic acid model [21]. Another study conducted by Xu *et al.* (2001) showed that all the three γ-OZ components had higher antioxidant activities against cholesterol oxidation than that of any of the four vitamin E components (α-tocopherol, α-tocotrinol, γ–tocopherol, and γ-tocotrinol) and that the highest antioxidant activity was found for the 24-methylenecycloartanyl ferulate [22].

Terada *et al.* (2003) in their study again showed that γ-OZ was able to inhibit isolated lipoxygenase with an IC_{50} of 25 μmol, whereas the ferulate esters in γ-OZ inhibited lipoxygenase with an IC_{50} of 15-34 μmol in the same model [23].

In vitro Anti-Inflammatory Activity

γ- OZ also inhibited isolated COX-1 and COX-2 with IC_{50}s of 13 and 38 μmol respectively. Ferulate esters in γ-OZ, e.g. cycloartenyl ferulate and stigmasterol ferulate inhibited COX-1 and COX-2 with IC_{50}s of 14-32 μmol [23]. It was not possible to comprehend the exact model for the study conducted by Terada *et al,* as only the abstract and tables were written in English.

In vivo Anti-Ulcer Activity

Itaya *et al.* (1976) in their study reported that γ-OZ, given at 1 to 100 mg/kg s.c. daily for five days, reduced the water-immersion stress ulcer index dose-dependently and slightly prevented the rate of increase in serum 11-hydroxy-corticosterone levels. These effects were prominent in adrenalectomized as well as sham operated rats. Thus, it apparently seemed that the anti-ulcer effect of γ-OZ is due to participation of the autonomic nervous system, but not the hypophysis-adrenal axis [24].

In male Wistar rats, a 8-day treatment with 100 mg/kg γ-OZ (s.c.), significantly reduced gastric ulcers, while 5-day pre-treatment exerted mild effects on ulcers induced by pylorus-ligation or stress atropine. Reduced serum gastrin levels were observed in rats with acetic acid induced gastric ulcers when γ-OZ was administered for 10 days (100 mg/kg body weight; subcutaneously) [25]. Treatment with reserpine prior to stress loading abolished the anti-ulcer effect of 100 mg/kg γ-OZ (s.c.) given for 5 days in stress ulcer. Administration of L-DOPA or 5-Hydroxy-Tryptophan, however, revealed a tendency toward restoration of the anti-ulcer effect. The possible mechanism pertaining to γ-OZ's anti-ulcer action might be the involvement of the monoaminergic neuron system.

Moreover, Mizuta *et al.* (1978) in their study reported that γ-OZ significantly inhibited tetragastrin-stimulated acid secretion, but was slightly effective as an inhibitor for histamine-stimulated acid secretion and non effective for carbachol-stimulated secretion [26]. The underlying mechanism might be mediated by the vagus nerve that induces gastrin release.

Another published report suggests that gastric lesions in responder mice induced by conditioned emotional stimuli was reduced by twice p.o. administrations at 6 hr intervals of γ-OZ at 200 and 500 mg/kg, oxazolam at 2 mg/kg and atropine at 1-10 mg/kg. In addition, the incidence of gastric lesions induced by Rapid Eye Movement (REM) sleep deprivation was also reduced by single administration of γ-OZ at 100 and 200 mg/kg and oxazolam at 5 mg/kg. Additionally, the facilitation of small intestinal propulsive activity in responder mice induced by convulsive electroshock was suppressed by γ-OZ at 100 and 200 mg/kg and atropine at 10 mg/kg [27]. Similar modulatory effect on gastrointestinal motility was observed in the dogs as well [28].

In vivo Anti-Hyperlipidemic Effects

There are several studies on humans and animals showing that γ-OZ has the potential of lowering low density lipoprotein cholesterol and total serum cholesterol and increasing the high density lipoprotein cholesterol to some extent either by influencing absorption of dietary cholesterol or by enhancing the conversion of cholesterol to fecal bile acids and sterols [29-33].

Sakamoto *et al.* (1987) studied the hypolipidemic effects of γ-OZ compared to cycloartenyl ferulate (CAF) in hyperlipidemic male Sprague Dowley (SD) rats. Rats (5-6/group) were fed a high cholesterol diet (1%) to induce hyperlipidemia [34]. γ-OZ was administered orally (100 mg/kg body weight) or IV (10 mg/kg body weight) once a day for 12 days along with cholesterol feeding. With the oral route, body weight, liver weight, serum lipids were unremarkable relative to controls on the high cholesterol diet for γ-OZ. Serum HDL-phospholipid levels were reduced only slightly relative to controls. Serum enzymes (AST and ALT), serum proteins and liver lipids were also unremarkable for γ-OZ. From an IV route, the only remarkable changes were in terms of reductions in serum triglycerides, low density lipoprotein (LDL), HDL/total cholesterol ratio serum non-esterified fatty acids, AST and serum albumin for γ-OZ. In summary, at relatively high oral doses γ-OZ does not affect liver function or serum lipids in hyperlipidemic rats.

In the above study, CAF was administered orally (100 mg/kg body weight) or IV (10 mg/kg body weight) once a day for 12 days with cholesterol feeding. In animals given CAF orally, serum lipids, serum proteins, serum enzymes, and liver lipids were all unremarkable. For CAF given IV, reductions in serum total cholesterol, phospholipids, HDL/total cholesterol (TC) ratio, LDH, triglycerides (TG), free cholesterol, non-esterified fatty acids, AST, ALT, serum total protein, albumin and liver phospholipids were observed. The study underlined the fact that γ- OZ and cycloartenol ferulate have an anti-hyperlipidemic action and this is more remarkable by intravenous than by oral administration, maybe due to a direct inhibition of lipid metabolism.

Nakayama *et al.* (1987) studied the hypolipidemic effects of γ-OZ in SD rats (10-12/group) with hyperlipidemia induced by 1% cholesterol diet for 12 days. γ- OZ was administered (100, 500 or 1000 mg/kg bw/day) in combination with the high cholesterol diet [35]. Body weights were unremarkable, but liver weights were around 10-15% higher on day 13 for the low dose (LD) and high dose (HD) groups, relative to controls (fed high cholesterol diet). TC was unremarkable on day 13, but around 25% lower on day 6 for all dose groups. Phospholipids were reduced on day 6 for the maximum dose (MD) and HD group, but were unremarkable on day 13. HDL-TC, HDL-phospholipids, serum protein and albumin were unremarkable. Serum free cholesterol was unremarkable except on day 13 where it was around 25% lower (HD only). Serum aspartate aminotransferase (AST) was slightly elevated but unremarkable whereas alanine transaminase (ALT) increased by around 65% (HD group only). Histological examination of the livers found steatosis but was unremarkable for the γ-OZ-fed animals.

In F_1B golden hamsters (16/group) fed with a hypercholesterolemic diet, 1% γ-OZ supplementation for 8 weeks resulted in reduced serum cholesterol (by 28%), sum of intermediate density lipoprotein (IDL), LDL and very low density lipoprotein (VLDL) cholesterol (by 34%), cholesterol absorption (by 25%), relative to controls [31]. Body weights, HDL cholesterol and TG serum levels were unremarkable along with unchanged liver and intestinal cholesterol synthesis.

In a separate experiment, 19 hamsters were divided into two groups and fed with chow-based diets containing 0.05% cholesterol with 10% coconut oil (control) and the control diet plus 0.5% γ- OZ for 10 weeks. Relative to the control, γ-OZ-treated hamsters had reduced plasma TC (44%, $p<0.001$), Non-HDL-C (57%, $p< 0.01$), and TG (46%, $p<0.05$) concentrations. Despite a 12% decrease in HDL-C ($p<0.01$), the γ-OZ-treated animals maintained a more optimum non-HDL-C/HDL-C profile (1.1±0.4) than the control (2.5±1.4; $p<0.01$). Aortic fatty streak formation, defined by the degree of accumulation of oil red O-stained macrophage-derived foam cells, was reduced by 67% ($p<0.01$) in the γ-OZ-treated animals [36].

In vivo Anti-Inflammatory Effects

Yasukawa *et al.* (1998) investigated the anti-inflammatory activity of γ-OZ and the steryl ferulate constituents of γ-OZ in the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced model of inflammation (ear oedema) in ICR mice [37]. Relative to γ-OZ, with an ID_{50} of 1.4 mg/ear (50% inhibition of oedema), the ID_{50}s for cycloartenyl ferulate, 24-methylenecycloartenyl ferulate, 24-methylcholesteryl ferulate and sitosterol ferulate ranged from 0.2-0.3 mg/ear.

Terada *et al* (2003) again examined the anti-inflammatory activity of γ-OZ in rats, wherein 7 rats /group were administered with γ-OZ (1, 10 and 100 mg/kg/day body weight; route not stated) for 19 days after inducing adjuvant-induced arthritis (unknown agent used) [23]. The paw-edema volume was significantly reduced from days 15-19 for the highest dose (HD), from days 17-19 for the medium dose (MD) and on day 19 for the lowest dose (LD).

A recent study conducted by Islam *et al.* (2008) investigated the effect of γ-OZ (50 mg/kg/day p.o.) on a mice model of induced colitis with dextran sulphate sodium (DSS) [38]. The results showed that γ-OZ could be a new potential therapeutic agent for gastrointestinal inflammatory diseases. The anti-inflammatory effect could be mediated by the inhibition of the inflammatory reactions exerted by tissue myeloperoxidase (MPO), pro-inflammatory cytokines and COX-2, NF-kappaB p65 nuclear translocation and inhibitory protein of nuclear factor-kappaB-alpha degradation.

Another recent study investigated the effects of the oral γ-OZ with ethanol (5.0g per kg) for 30 days to c57BL mice on ethanol-induced liver injury [39]. Preventions of ethanol-induced liver injury by γ-OZ were reflected by markedly decreased serum activities of plasma aspartate aminotransferase, alanine aminotransferase and significant decreases in hepatic lipid hydroperoxide and thiobarbituric acid reactive substances (TBARS) levels.

Another recent notable finding by *Oka et al.* (2010) revealed that IgE-targeting therapy by intra-dermal γ-OZ injection in SD rats with anti-DNP IgE could provide significant progress in the treatment of allergic inflammation [40]. The mechanism involved might be the attenuation of the passive cutaneous anaphylaxis (PCA) reaction induced by DNP-HAS and inhibition of the degranulation of DNP-IgE sensitized RBL-2H3 mast cells stimulated with anti-DNP-HAS. However, further investigation is necessary to address the IgE specificity of γ-OZ, as well their effects in animal models of allergic diseases.

Effects in Type II Diabetes

Ohara *et al.* (2009) in their study attempted to positively correlate serum adiponectin concentrations with insulin sensitivity in mice and observed that serum adiponectin concentrations were increased by γ-OZ administration [41]. To investigate the underlying mechanisms of adiponectin secretion, the effect of γ-OZ on adiponectin secretion in the NF-kappaB activation state was again investigated and it was observed that γ-OZ might regulate adiponectin secretion by the inhibition of NF-kappaB activation. Hence, γ-OZ might be effective for ameliorating type 2 diabetes, although more validated data is warranted on the same.

CNS and Behavioral Effects

Experimental animal models have been used extensively in psychopharmacology mostly in relation to the success or

failure of a given model in predicting the clinical potency of γ-OZ. The potential effects of γ-OZ (IP) and cycloartenyl ferulate (CAF; IP), a constituent of γ-OZ (around 20-30% w/w) on the central nervous system (CNS) and behaviour were investigated in male Slc:ICR mice, male Wistar rats and mixed bred cats [42].

In the experiment examining the effects of single administration of γ-OZ or CAF on pentylenetetrazol (PTZ: 140 mg/kg; SC)-induced convulsions, one hour after injection of the convulsant, mice (10-20/group) were administered one of the following treatments: vehicle only (controls), CAF (1, 10, 100 or 1000 mg/kg; IP), diazepam (2 mg/kg; IP), CAF (10, 30, 100 or 300 mg/kg; PO) or γ-OZ (1000 mg/kg; PO). Time for the onset of convulsions and time to death were recorded. Given orally, the effect of γ-OZ was insignificant with respect to the seizure onset time and time to death. CAF (PO), on the other hand, increased the time to seizure onset in a dose-dependent manner, but had no effect on time to death. Given IV, CAF increased seizure onset time at the LD and the HD, but had no effect on time to death at either dose.

The conflict behaviour experiment involved a single administration of γ-OZ (30, 100 or 300 mg/kg; IP) or CAF (3, 10 or 30 mg/kg; IP) to mice 1 h before testing in a 1 h session of a safe period (6 min) allowing mice to press a lever to eject pelleted food (rewarded every 90 s) alternated with a warning period (buzzer tone sounded), whereby every 5th press produced pelleted food followed by a 50-120 V AC foot shock. Analyses of the number of lever presses in the safe versus the warning periods showed a significant reduction with γ-OZ administration during the safe period at the LD and the HD, with no changes being observed for the warning period.

Andoh *et al.* (1994), in their study, reported the effects of oral γ-OZ on electroencephalographic (EEG) activity in rabbits [43]. Rabbits were instrumented with screw electrodes under anaesthesia into the cerebral cortex and motor, visual and sensory cortices. One week later, EEG arousal response was elicited with and without sound stimulation (for 10 s) and recorded from the cerebral cortex, amygdaloid nucleus and the hippocampus. γ- OZ (50 mg/kg; PO) desynchronised EEG activity 10-15 min after administration for 30-40 mins and again from 60 mins post-dose for 120 mins. This was interpreted as inducing an arousal-resting-arousal EEG pattern. With sound stimulation, the EEG response was insignigicant relative to control group. In a separate experiment, the effects of γ-OZ on the duration of the amygdaloid afterdischarge induced by electrical stimulation of the amygdaloid nucleus were examined in anaesthetised and immobilised rabbits. γ- OZ (50 mg/kg; PO) was found to prolong the duration of the after-discharge, beginning 45 min post-dose.

In cats, EEG activity after a single IV administration of γ-OZ (dose not specified) was monitored and showed low amplitude fast waves starting at 40 mins post-dose, lasting from 12 to 110 mins. Higher dose of γ-OZ increased the duration of the fast wave period [44].

Another study conducted by Kaneta *et al.* (1979) revealed that γ-OZ (100 mg/kg; SC) administered once daily for 1, 5 or 10 days increased nor-epinephrine levels in the brain of rats [45]. Hence, it is likely that successive doses of γ-OZ increase brain nor-epinephrine by inhibiting the degradation or release of nor-epinephrine.

In vivo Chromosomal Effects

Tsushimoto *et al.* (1991) investigated the effects of γ-OZ on chromosome aberration in bone marrow cells from SD rats given the compound orally at 40, 400 and 4000 mg/kg as a single or repeat-doses (once daily for 5 days) [46]. No cases of mortality were observed in the single dose group and the data indicated that γ-OZ did not induce chromosome aberration. Similarly, in the repeat-dose study, no dose-dependent effects on chromosomal aberration were observed. However, one animal showed decreased body weight and "languid" behavior on day 4 and died on day 5 at the HD. However, subsequent necroscopy revealed an intubation error into the lung. It was not stated whether any other toxic signs were observed in any of the other animals.

In vivo Carcinogenicity Studies

The carcinogenic potential of oral γ-OZ has been investigated in two long-term studies in B6C3F1 mice and F344 rats by Tamagawa *et al* (1992) [47, 48].

Carcinogenicity Studies in Mice

B6C3F1 mice in groups of 50 males and 50 females were fed a diet containing 0 (control), 200, 600 or 2000 mg γ-OZ/kg body weight/day for 78 weeks. No treatment-related changes were observed in general condition, body weight, food consumption, mortality, organ weight or haematology. Histopathological examinations showed tumour incidence in all the groups, including the control group. Microscopic examinations were conducted on tissues from various body organs for the controls and the HD group, and the mesenteric lymph node, thymus, spleen, liver and kidneys of surviving animals at the LD and MD were also examined for malignant lymphoma and/or leukaemia. In the control and 2000-mg/kg groups, relatively high tumour incidences were observed in the liver of males and in the haematopoietic organs of females. However, there was no statistically significant difference in the incidence of any tumours between the control and the 2000-mg/kg groups. The findings indicated that under the experimental conditions described above, γ-OZ was not carcinogenic in B6C3F1 mice.

The anti-carcinogenic potential of CAF was examined by Yasukawa *et al.* (1998) in the female ICR mice (15/group) using a two-stage model of skin carcinogenesis with 7, 12-Dimethylbenz[a]anthracene (DMBA) as a single topical dose (50 μg) to the back as an initiator of skin tumours, followed by the promotion with topical TPA (1 μg) twice/week in week 2 [37]. Topical CAF was applied at the dose of 2 μmol/mouse was applied 30 min before each DMBA administration for 20 weeks. The results of the study indicated that CAF reduced the percentage of tumour bearing mice from 93% to 20% at week 20, although no reports of statistical analyses were presented. The number of tumours/mouse also decreased by a similar factor.

Carcinogenicity Studies in Rats

Using the same experimental protocol described by Tamagawa *et al* (1992), Fischer (F344/DuCrj) rats (50/gender/group) were fed a diet containing γ- OZ at concentrations of 200, 600 or 2000 mg/kg/day for 2 years. Body weights were unremarkable relative to controls at all doses. While survival times were significantly reduced for females in the LD and MD groups relative to controls, the effect was not seen at the HD and not in males at the equivalent doses. Minor body weight reductions were observed for males from around week 60 at the HD, but the terminal mean body weight gain of males was unremarkable. Although females in the highest dose group (2000 mg/kg body weight) showed a slight decrease in body weight at 104 wk, there were no treatment-related changes at necroscopy, in terms of food consumption, mortality, organ weight or haematology. Histopathological examination showed tumour incidence in all groups irrespective of males and females, including the control group. In the control and 2000-mg/kg groups, high tumour incidences were observed in the testes, pituitary and thyroid of males, and in the pituitary, uterus and mammary gland of females; however, there was no significant increase in the incidence of any tumours between the control and the 2000-mg/kg groups. The findings indicated that under these experimental conditions γ- OZ was not carcinogenic in F344 rats.

The modifying effects of γ- OZ on the promotion stage of carcinogenesis were investigated using several two stage carcinogenesis models in rats [49]. In a multi-organ carcinogenesis model, male F344 rats were treated twice with 2,2'-dihydroxy-di-n-propylnitrosamine (DHPN; 1000 mg/kg body weight intraperitoneally), twice with N-ethyl-N-hydroxyethylnitrosamine (EHEN; 1500 mg/kg body weight intragastric), and three times with 3,2'- dimethyl-4-aminobiphenyl (DMAB; 75 mg/kg body weight; subcutaneously) at 3-4 day intervals during the three week initiation period. One week after initiation, rats were started on a diet containing 1% γ- OZ or basal diet alone for 32 weeks. Control groups (10/group) were fed 1% γ- OZ or basal diet only. Animals were examined histopathologically at termination. Liver adenoma in carcinogenesis induced rats decreased from 64% in animals fed with the basal diet to 38% in γ- OZ –treated animals ($p<0.05$), but when lung lesions were examined, the incidence of lung carcinoma increased from 8% (1 rat) to 54% (8 rats) and multiplicity also increased from 0.1/rat to 0.6/rat. An increase in the multiplicity (but not incidence) of lung adenoma was also observed in γ- OZ-treated rats. Esophagus, colon, pancreas, kidney and thyroid lesion development was not influenced by the compound.

In a γ- OZ dose response experiment using DHPN as an initiator in the drinking water, enhancing effects on lung were observed at a dose of 1% but not at 0.5% or lower. Finally, examination of the modifying potential of 1% γ- OZ on mammary carcinogenesis in female Sprague Dawley rats pre-treated with a single intragastric dose of DMBA (50 mg/kg body weight) for 35 weeks revealed no significant differences in the final incidences and multiplicities of mammary tumors, but the average tumor diameter was significantly reduced. The survivorship curve for the γ-OZ-treated rats was >10% higher than controls from week 22 of the experiment and around 50% higher at study end. In summary, the above data suggest that at relatively high dietary intake levels γ-OZ may enhance tumour promoting effects in chemically-induced- multi-organ carcinogenesis and lung cancer models in rats, but γ-OZ *per se* did not initiate carcinogenesis.

PHARMACOKINETICS OF γ-OZ

γ- OZ, being a complex multi-constituent substance, data describing the pharmacokinetics of its individual constituents has been reported.

Fujiwara *et al.* (1980) in their study in rabbits showed that the blood level of radio-labeled 'γ-OZ' was very low following an oral administration (40 mg/kg body weight) and was undetectable in blood 2 h post-dose [30]. The total radioactivity appearing in urine 48 h post-dose was 6.4% of the administered amount. The principle metabolite found in the blood was ferulic acid (FA). However, in urine, FA, vanillic acid, acetovanillone, hippuric acid and the glycine conjugate of vanillic acid were detected. The underlying mechanism might involve the poor absorption, rapid tissue distribution and extensive metabolism of 'γ- OZ', probably in the liver.

Fujiwara *et al.* (1983) investigated the absorption and metabolism of ^{14}C-labelled 'gamma oryzanol' (50 mg/kg bw; PO) after oral administration in male SD rats [31]. Analyses of the urine and faeces over the next 5 days showed that 9.8% of the ingested radioactivity was excreted in the urine over the first 72 h and faecal excretion was 84.5% for the first 48 h. 94% of the urine and 93% of the faecal radioactivity was excreted within the first 24 h and radioactivity in the blood peaked at 4 h and disappeared by 48 h post-dose. An analysis of the urine metabolites revealed no 'γ-OZ', but around half of the 'γ-OZ' dose was accounted for as the sulfated form of FA and significant proportions of hippuric acid, m-hydroxyphenylpropionic acid and dihydroferulic acid were also detected along with small quantities of m-coumaric and m-hydroxyhippuric acid.

Fujiwara *et al.* (1982) conducted another set of experimental studies using unlabelled 'γ-OZ', most likely cycloartenyl ferulate or β-sitosteryl ferulate, wherein rabbits (5 males) and beagle dogs (3 males) were administered 'γ-OZ' at 25, 50 and 100 mg/kg body weight (PO) after a 20-24 h fasting [50]. Blood was analysed at regular intervals for FA till 24 h post-dose. A prominent dose-dependent relationship was observed in both the species with peak plasma FA levels occurring at 2 h in rabbits and 1-1.5 h in dogs. The maximum plasma levels of FA were 100 ng/mL and 200 ng/mL in dogs and rabbits respectively. At 24 h post-administration, plasma FA returned to baseline levels in rabbits and dogs.

Another study by Tsushimoto *et al.* (1991) reported the maximum plasma concentration (C_{max}) after a single oral consumption of 300 mg 'γ-OZ', actually β-sitosteryl ferulate, to be around 40 ng/mL in humans [46].

CLINICAL DATA

The clinical studies for γ-OZ are summarised chronologically in Table **1**.

Table 1. Summary of Clinical Trials on γ- OZ

Study Design (Reference)	Subject Details	Treatment Details (Dose and Route)	Endpoints	Key Outcomes	ADRs
Controlled, crossover, 1 day Shimomura *et al.* (1980) [51]	n=7 (both sexes) Aged 20-45 yrs. Patients with primary hypothyroidism	Oral; 300 mg (single dose)	T4-I, T3 and TSH	25% reduction in serum TSH at 2-4 h post-dose, possibly by a direct action on the hypothalamus	None reported
Controlled, cross-over 1 day Shimomura *et al.* (1980) [51]	n=7 (both sexes) Aged 20-45 yrs Patients with primary hypothyroidism	Oral; 300 mg (single dose) and TRH (500 μg; IV)	TSH	No significant differences	None reported
Controlled, 7 days Shimomura *et al.* (1980) [51]	n=8 (both sexes) Aged 20-45 yrs Patients with primary hypothyroidism	Oral; 300 mg/day	Serum TSH	Unclear	None reported
Open labeled, 4 weeks Arai (1980) [52]	n=19 (sex unclear) Aged 25-74 yrs Patients with chronic gastritis and 62% also neurotic	Oral; 300 mg/day	Serum gastrin, frequency of symptoms	serum gastrin "75% effective" in symptom reduction	None reported
Controlled 4 weeks Arai (1980a) [53]	n=13 (sex not stated) Patients with chronic gastritis	Oral; 300 mg/day	Serum gastrin, secretin, glucagon, catecholamines, prolactin, cortisol.	Reduced serum gastrin	None reported
Open labeled, 8 weeks Ishihara *et al* (1982) [54]	n=40 (sex not stated) experiencing aging syndromes and with climacteric disturbance	Oral; 300 mg/day	Effect on climacteric disturbance	✓ 90% of the cases improved generally; ✓ Significantly reduced total cholesterol (TC), triglyceride ✓ Increased HDL-cholesterol noted in cases with hyperlipidemia ✓ lipid peroxides levels significantly recovered	None observed
Open labeled, 8 weeks Ishihara *et al* (1984) [55]	n=40 (females) Aged ≥40 yrs Patients with climacteric disturbances	Oral; 300 mg/day in 3 divided doses	Serum lipids, serum lipid peroxide, frequency of menopausal symptoms, menopausal index, liver enzymes, haematology	✓ Reduced serum lipid peroxides (31%; week 4) ✓ Reduction in symptoms (75-100%) ✓ Reduced menopausal index (improvement) in 34/40 ✓ Reduced TG (14%)	None observed
Open labeled, 4 weeks Kawamoto *et al.* (1985) [56]	n=24 (sex not stated) 7 patients with gallstones, 3 with hyperlipidemia and 10 normal subjects	Oral; 600 mg/day	Serum apolipoprotein A-I, lipids	Reduced serum apolipoprotein A-I	Not stated
Randomized controlled, (13 weeks) Yoshino *et al,*1989 [57]	n= 80 Hypercholesterolemic patients	Oral; 300 mg/day	TC, LDL-C, TG	Reduced TC, LDL-C, TG	None observed

Table 1. contd….

Study Design (Reference)	Subject Details	Treatment Details (Dose and Route)	Endpoints	Key Outcomes	ADRs
Open labeled, 16 weeks Sasaki *et al.* (1990) [58]	n=20 (11 males; 9 females) Mean age 51 yrs, Patients with schizophrenia and dyslipidaemia taking neuroleptic medication	Oral; 300 mg/day in 3 divided doses	Serum lipids (TC, LDL, HDL, TG, LDL/HDL), apolipoproteins	✓ Reduced TC (6%) at weeks 8 and 14% at week 12 ✓ Reduced LDL (19%; week 12 only) ✓ Reduced HDL (6%; at week 8 only) ✓ Reduced LDL/HDL (11%; at week 4 only) ✓ Reduced apolipoproteins A-II, B, C-II and B/A-I ratio at weeks 8 and 16 (by 9-15%)	None observed
Open labeled, 8 weeks Bucci *et al.* 1990 [59]	n= not reported (males) body builders	Oral; 30 mg/day	body weight and strength	Increase in body weight and strength	Not reported
Open labeled, 2-6 months Fujiwaki & Furusho (1993) [60]	n=20 (8 males; 12 females) Aged 2-15 yrs Patients with atopic dermatitis In combination with existing medications including steroids	Topical; 0.5% added to bath once a day	Symptom score, IgE, eosinophil count, specific IgE antibodies	✓ Reduced total symptom score ✓ Reduced eosinophil count ✓ Reduced specific IgE in 2/6 substances tested	None observed
Randomised, double blind, placebo controlled 9 weeks Fry *et al.* (1997) [61]	n=22 (males) Aged 19.8 ± 0.87 yrs Weight-trained subjects	Oral; 500 mg/day and 9 week resistance training programme	Testosterone, β-endorphin, cortisol, estradiol, insulin, hematocrit, albumin, plasma lipids, calcium and magnesium Strength tests, lactate pre- and post-exercise.	No treatment-related effects, no influence on performance or related physiological parameters	None observed
Randomised, double blind, placebo controlled, cross-over 3.5 weeks Westsrate & Meijer (1997) [62]	n=100 (50 males, 50 females) Aged 18-65 yrs Healthy subjects	Oral; 2.7 g/day "phytosterol esters from RBO" (1.7 g/day sterols)	Lipids, liver enzymes, bile acids, sterols, fatty acids, carotenoids.	✓ Reduced TC (8.3%) ✓ Reduced LDL (13%) ✓ α + β-carotene (8%)	Not reported
Randomised 4 weeks Berger *et al.* (2005) [63]	n=30 (males) Aged 40-65 yrs Total cholesterol 5.1-8.4 mmol/L BMI < 28 kg/m^2	Oral; 50 mg/day or 800 mg/day in diet	Serum lipids (TC, LDL, HDL, LDL/HDL, VLDL	✓ Reduced TC (6%), LDL (11%), HDL (8%), LDL/HDL (19%) and VLDL (56%)	None observed

* TC: total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL: High density lipoprotein; VLDL: Very Low density lipoprotein; TG: triglycerides

MISCELLENOUS USES OF γ-OZ

γ- OZ is used as a sunscreen agent on account of its protective role in UV-light induced lipid peroxidation. A number of patented cosmetic sunscreen compositions containing γ- OZ have been reported [64, 65]. Ferulic acid esters like γ-OZ are capable of stimulating hair growth and prevent skin

aging by accelerating the cell differentiation and reducing the wrinkles in aged women, thereby protecting them from oxidative damage [66, 67].

In spite of the fact that γ- OZ is widely employed as an antioxidant in the cosmetic industry, only a single literature report is available about its modulating effect on sebaceous gland secretion after topical application [68].

Solubilization of γ- OZ into medicinal drinks serve as revitalizing tonics and is achieved by using sucrose fatty acid ester and ethoxylated HCO [69, 70].

Moreover, γ- OZ is widely employed as an anabolic agent by bodybuilding athletes because of its potential to increase testosterone production and stimulate human growth hormone release [71, 72].

ADVERSE REACTIONS

γ- OZ has been shown to be very safe with no major side effects being reported in either animal or human studies [73]. However, the International Adverse Reaction Reports in the World Health Organization (WHO) database indicates that out of the 7 cases of adverse reactions reported, causality was problematic in six reports, where other drugs were administered along with γ- OZ. Erythematous rashes were observed in one such case with 75 mg/day oral γ- OZ when co-administered with betahistine hydrochloride and bisbutamine for 14 days. Another report suggests erythroderma related adverse reactions with 450 mg/day oral γ- OZ co-administered with etizolam for 6 months. Incidence of rashes and eosinophilia were notable in another case when the treatment regime with γ- OZ included tosufloxacin tosylate for 2 days and lenampicillin hydrochloride for 12 days, although the exact dose was unclear. Another instance of breast pain was observed with 1 g/day oral γ- OZ along with pyridoxine hydrochloride/cyanocobalamin, enzymes, dried yeast, tolbutamide, cyclandelate, sanactase, proctase, meicelase, olipase-2S and pancreatic digestive enzyme for 76 days. Nocturia was observed in another patient with 150 mg/day oral γ- OZ with domperidome and kallidinogenase for 2 days. In another such case, extra-pyramidal disorder was reported with 20 mg/day γ- OZ for 1 day, although the symptoms abated upon withdrawl. The reaction, however, resurfaced upon rechallenge, thereby citing a good evidence of causality in this regard. Thus, to summarize, γ- OZ, as a dietary supplement, is considered adequately safe with no significant side effects being produced in experimental and clinical studies till date [74, 75].

REGULATORY SCENARIO

The global approach to regulating and marketing nutraceuticals is notably heterogeneous on account of the challenges in classifying these products and the absence of a suitable regulatory framework [76]. As per the Australia New Zealand Food Authority Annual Report 1999-2000, in the US, unlike 'vegetable oil phytosterol esters', γ- OZ does not have a Generally Recognized as Safe (GRAS) 1 status, which is one of four legal categories set up by the US Congress under the 1958 Food Additives Amendment to the Federal Food, Drug, and Cosmetic. Furthermore, the database of the Centre for Food Safety and Applied Nutrition of the US Food and Drug Administration (EAFUS – 'Everything Added to Food in the United States') does not contain any listings for γ- OZ. Additionally, γ -OZ does not appear in the US Environmental Protection Agency's database of existing chemicals, the Toxic Substances Control Act Chemical Substance Inventory. Moreover, since γ- OZ is not approved by the Flavour and Extract Manufacturers' Association (FEMA), it cannot be used as food additives or pharmaceuticals in the US. However, in the US, γ- OZ is widely used as a sports supplement, as well as for reducing cholesterol, despite the lack of any meaningful evidence supporting its use in any of these conditions [77].

Currently, γ -OZ has also been approved in Japan for several conditions, including menopausal symptoms, mild anxiety, stomach upset, and high cholesterol and has been listed as an "antioxidant" under the list of chemical composition of food additives [77].

CONCLUSIONS

Considering an emerging public and scientific interest in the use of phytochemicals derived from dietary components for their therapeutic usage, a wide spectrum of beneficial activity for human health has been advocated for γ- OZ, because of its strong antioxidant activity, explained by the free radical scavenging activity. However, these studies did not produce unequivocal conclusions and have been run on a wide variety of animal species very different from each other, and using different methodologies and targets. Hence, wider randomised controlled clinical trials would be useful to confirm the potential of γ- OZ's reducing properties in plasma TC, LDL-C and TG and its capacity to raise HDL-C. Additionally, further studies at higher dose levels would provide more confidence in both the safety of this product and in its capacity of maintaining long-term reductions in plasma cholesterol levels, thereby subsequently benefitting on the cardiovascular outcomes in human subjects.

Other potential range of therapeutic properties exhibited by γ- OZ include anti-carcinogenic, anti-inflammatory, anti-diabetic, anti-ageing, neuroprotective and hepatoprotective effects, most of which can be attributed to its potent anti-oxidant capacity. Moreover, γ- OZ works well in all herbal antioxidant formula, vitamin and herbal health supplements that benefit our body's immune system to a considerable extent. In the light of these potential benefits, γ- OZ is definitely a promising addition to the therapeutic armamentarium against various oxidative stress related diseases.

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