Chapter

Functional and Therapeutic Potential of γ-Oryzanol

Aasiya Sulaiman, Aisha Sulaiman, Mehtap Sert, Mohammed Safwan Ali Khan and Mansoor A. Khan

Abstract

This chapter summarizes the entire literature available on the nutritional value and diverse therapeutic potentials Gamma-oryzanol, a nutraceutical obtained from rice brain oil, composed of a mixture of γ - oryzanol, a mixture of ferulic acid esters of phytosterols and triterpenoids, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate. In brief, the review covers the aspects such as the antioxidant mechanisms, effects on immune system, lipid disorders, diabetes, obesity and inflammation with the details of preclinical experiments, models and observations. Among the other highlights are the hepatoprotective, neuroprotective role in various neurological disorders such as Alzheimer's, anxiety, Parkinson's disease and wound healing effects. An overview of the sources, chemistry, physicochemical properties, pharmacokinetics and toxicity studies are also included.

Keywords: Gamma oryzanol, rice bran oil, pharmacological activities, nutraceutical, organoprotective

1. Introduction

Rice is a staple food in many countries around the world. Milling of rice gives an 8% byproduct, rice bran [1]. Crude rice bran oil is obtained by different methods. According to Godber and Xu the most efficient way to extract rice bran oil is superficial fluid extraction technology [2]. Rice bran oil contains the naturally occurring nutritive and antioxidant phytochemical, γ- oryzanol [1]. It is one of the phytochemicals present in high amounts in rice bran [3].

The molecular formula of γ- oryzanol is $C_{40}H_{58}O_4$ and its melting point is 137.5-138.5°C. It is a white crystalline powder. γ- oryzanol is insoluble in water. However, it is slightly soluble in diethyl ether and n-heptane. Previously, γ-oryzanol was thought to be a single compound [1]. It was later discovered that γ- oryzanol is a mixture of phytosterylferulates of ferulic acid esterified with phytosterols including sterols (campesterol, sitosterol, and stigmasterol) and triterpene alcohols (cycloartenol and 24- methylenecycloartenol) [3]. The structure of gamma oryzanol is shown in **Figure 1**.

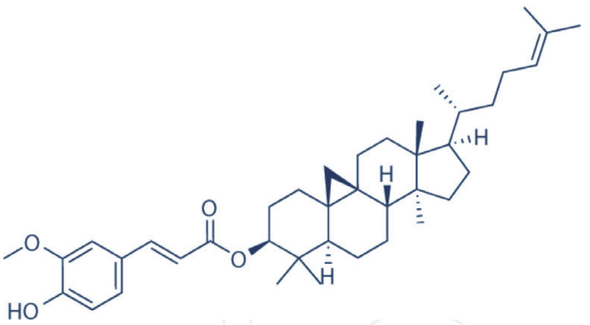

Figure 1.
Structure of gamma Oryzanol.

2. Pharmacokinetics and toxicity of γ- oryzanol

An experiment carried out by Fujiwara *et al*. revealed that γ-oryzanol is readily absorbed by the intestine and is found in high concentrations in the plasma after 1 hour of oral administration [4]. In 2016, Seol-Hee, *et al.* conducted a toxicity study using male and female Sprague–Dawley rats, 1000 and 2000 mg/kg body weight per day were administered to 5 weeks old rats for 90 days. It was observed that the rate of absorption of γ- oryzanol was low and most of it was excreted. The study concluded that there were no adverse effects on administration of 1000 and 2000 mg/kg body weight per day of γ- oryzanol [5].

3. Therapeutic potentials of γ- oryzanol

In the recent times, many researchers have conducted both *in-vitro* and *in-vivo* experiments to explore the bioactivities of γ- oryzanol. Some of these investigations have revealed promising role of γ- oryzanol in certain medical conditions. The pharmacological properties of γ- oryzanol have been summarized in **Table 1** and depicted in graphical abstract, **Figure 2**.

3.1 Antioxidant activity

A compound that halts the effect of free radicals towards cells is referred to as an antioxidant. Free radicals are unstable molecules generated by a reaction to environmental and caused by other factors. Antioxidants can be natural or artificial in nature. Degeneration of lipids by means of oxidation is referred to as lipid peroxidation. In general, free radicals tend to "steal" electrons from lipids in cell membranes, ultimately leading to cell damage which activates the free radical chain mechanism.

The antioxidant activity of γ- oryzanol was assessed against hydroxyl radicals generated by Fenton reaction, and superoxide radicals generated by auto oxidation of $FeCl_2$. The free radical scavenging efficacy of γ - oryzanol was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay while the lipid peroxidation preventive activity was screened by 2,2-azobis(2,4-dimethylvaleronitrile) AMVN assay. Finally, the antioxidant potential of γ- oryzanol within oil samples was analyzed by conductometry in accelerated oxidation conditions. IT, the time taken to achieve sharp increase of conductivity was measured in oils in the presence and absence of γ- oryzanol butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) at different concentrations in a study conducted by Juliano *et al.* [6].

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of γ- oryzanol	Results	Reference
1.	Antioxidant activity	Hydroxyl radicals generated by Fenton reaction	1.65 μM 16.5 μM	No interaction with hydroxyl radicals No interference in Fenton reaction	Juliano et al. [6]
		Superoxide radicals generated by autooxidation of $FeCl_2$	10 μM	Did not scavenge superoxide radicals	
		DPPH assay	10,20,30,40,120,240 μM	Dose-dependent DPPH scavenging but weaker than activity. A-tocopherol dose-dependent reduced rate of peroxidation	
		AMVN initiated lipid peroxidation	50 & 100 μM	50 μM of γ- oryzanol more effeicient than 10 μM α-tocopherol	
		Conductometric evaluation of anti-oxidant activity in oils	2.5-20 mmol/kg oil	Progressive ↑ of AI values of all samples max. Effect at 10 mmol	
2.	Antihypercholeterolemic activity		p.o. 0.5%		Wilson et al. [7]
		Non-purified hypercholesterolemic diet induced lipid disorder (HCD-10% coconut oil +0.1% cholesterol for 2 weeks) in golden Syrian hamsters.	Plasma lipids	RBO↓plasma TC, LDL&VLDL(64% &70%); ORY(70%&77%); ferulic acid & ORY (13%-24%) RBO↑ HDL-C conc. (10%-20%) RBO(53%)&ORY(65%) diets↓ plasma TG conc.	
			Plasma vitamin E	No significant difference exhibited on plasma α &γ - tocopherol	
			Plasma lipid hydroperoxides	ORY(73%)↓ plasma lipid hydroperoxides by 46%)	
			Aortic cholesterol	RBO (73%) & ORY (46%) diet ↓ aortic cholesterol ester accumulation	
			Fecal neutral sterol content	ORY excreted significantly more coprostenol(127%) &(120%)	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of *γ*- oryzanol	Results	Reference
3.	Anti-diabetic activity	Wistar rats Streptozotocin & nicotineamide induced T2D	Streptozotocin – 45 mg/ kg + nicotineamide 200 mg/kg *γ*- ORY (5.25 gm)		Cheng *et al*. [8]
			Plasma insulin conc.	AUC for insulin was ↓ by ORY	
			Liver TG&TC	TG ↓ & TC ×	
			Fecal cholesterol		
			Weight gain & food intake	×	
			Plasma glucose	×	
			Insulin sensitivity		
			Plasma TG & NEFA conc.	ORY & PO ↓ TG conc. & NEFA ↓	
			Plasma cholesterol	ORY ↓ LDL-C conc. While ↑ HDL-C. TC/ HDL-C ratio ↓	
			Fecal neutral sterol& bile acid contents	Significant ↑	
4.	Effect on male gonads		33 mg/ mw p.o./ day for 30 days		Escobar *et al.* [9]
	Testicular degeneration induced by scrotenone al insulation in rams		Testicular steroidogenic activity	×	
			Sperm membrane integrity	The semen integrity was affected	
			Sperm morphology & motility	↑ in sperm motility	
			Semen	×	
			Oxidative stress	↓ in ROS by 26%	
			Serum testosterone	×	
			Testicular morphology	Morphology of testes was affected	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of γ- oryzanol	Results	Reference
5.	APAP induced liver injury				Shu *et al.* [10]
	Liver injury induced by acetaminophen		Cell viability and toxicity in HL7702 hepatocytes	↑ cell viability, ameliorated toxicity	
			Expression of Nrf2 i-NOS and COX_2	↑ Downregulated	
			Levels of proinflammatory factors – TNF-α, IL-1B, IL-6, NO	↓	
			Serum ALT, AST& LDH levels	↓	
			Activity of caspase −3, −8 and − 9	↓ dose dependent	
			BCl_2 and Bax protenone ein analysis	↓ ↑	
			Histopathology and immunohistochemistry.	ORY attenuated intra tissue hemorrhage & inflammatory cells infiltration	
				ORY modulated AMPK/GSK3β/Nrf2 and NFkB signaling pathways	
6.	Et-OH induced liver injury				Chotimarkorn and Ushio [11]
			Serum AST & ALT	↓	
			Hepatic lipid peroxidation	↓	
			TBARS	↓	
			Glutathione	↓	
			SOD	↑	
7.	CCl_4 induced liver injury				Gomes *et al*. [12]

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro*/ *in vivo*)	Parameters of *γ*- oryzanol	Results	Reference
			Hepatic function parameters	γ-ORY supplementation & treatment with silymarin stopped ↑ in AST, ALT, ALP, GGT and LDH activities & ↓ the levels of bilirubin	
			Oxidative parameters TBARS NPSH AA	 γ-ORY supplementation & treatment with silymarin ↓ lipid peroxidation γ-ORY supplementation & treatment with silymarin ↑ NPSH levels Treatment with silymarin ↑ AA levels	
			Inflammatory parameters TNF-α, IL-1β, IL-6, TGF- β1& IFN-γ levels. MCP- 1 MPO activity NO levels	 γ-ORY supplementation & treatment with silymarin reversed the ↑ in TNF-α, IL-1β, IL-6, TGF- β1& IFN-γ levels ↓ by treatment with silymarin ↓ by γ-ORY supplementation and treatment with silymarin ×	
			Apoptopic parameters	Caspase 3 and 9 activity ↓ **γ**- ORY supplementation and treatment with silymarin	
9.	Effect on hypoadiponectinemia		Serum adiponectin levels	↑ adiponectin levels	Nagasaka *et al.* [13]
10.	Effect on Immune system				Fujimoto *et al.* [14]
			Release of β-hexoaminidase by anti-DNP IgE sensitized RBL-2H3 cells post DNP-HSA stimulation.	↓ in dose dependent manner	
			PCA reaction test and the IgE detection test by ELISA	× mast cell degranulation	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of γ- oryzanol	Results	Reference
			Electrophoretic mobility	×	
			NFκB activity	CAF↓	
11.	Anti-inflammatory activity				Rao *et al.* [15]
			Reactive oxygen species	RBO-N generated lower levels of O2 and NO by 51 and 45%	
			Eicosanoids	γ –ORY ↓ secretion of pro-inflammatory eicosanoids by macrophages	
			Cytokines	↓ secretion of pro-inflammatory ↑ secretion of anti-inflammatory	
			Lysosomal enzymes	RBO fed group secreted lower levels of collagenase, elastase and hyaluronidase by 42, 43 and 55%	
12.	Anti-parkinsonism activity				Araujo *et al*., [16]
			Dopamine conc. by HPLC	↑ by ORY	
			Survival rate	↑	
			Locomotor	ORY abolished locomotor deficit caused by rotenone	
			AchE activity	Effect of rotenone on AchE activity was overcome by ORY	
			Cell viability	↓ by ORY	
			Mitochondrial viability	↓ by ORY	
			Resazurin reduction assay	↑	
			Antioxidant defenses MDA, SOD, CAT & GST	↓ inhibition of MDA, SOD, CAT & GST.	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro*/ *in vivo*)	Parameters of *γ*- oryzanol	Results	Reference
13.	Anti-anxiety activity				Akter *et al*. [17]
			Food intake and body weight	↓ in food intake and blocked the stress-induced reduction of body weight gain.	
			CRST (OFT)	*γ*- ORY ↓anxiety like behavior	
			CRST (EPM)	*γ*- ORY showed ↓ in anxiety	
			Serum corticosterone	×	
			BMA	Examination of amygdala, hippocampus and cerebral cortex showed that *γ*- ORY has anxiolytic effect	
14.	Anti-Alzheimer activity				Jha and Panchal [18]
			Alamar blue assay	↑cell survival at 100 nM, 1 μM, 100 μM	
			Total arm entry	ORY ↑ total arm entry	
			Correct arm entry	ORY ↑ correct arm entry	
			Reference memory error	↓ %RME by ORY	
			Memory score	↑ with DONO & ORY	
			MDA	↓ by ORY	
			GSH	↑ by ORY	
			CAT	↑ by ORY	
			BMA	ORY ↑ BMA	
			Brain AchE	↓ AchE activity	
		CRP		DONO & ORY ↓ CRP	

S. No	Bioactivity	Experimental model/ Assays/ (*in vitro/ in vivo*)	Parameters of *γ*- oryzanol	Results	Reference
15.	Anti-obesity activity		0.5 w/w		Francisqueti *et al*. [19]
			Caloric Ingestion	×	
			Inflammatory parameters	↓	
			Renal function parameters	*γ*- ORY restored renal function in HSF/ HSF + *γ*- ORY group	
			Redox state parameters	*γ*-ORY ↑ hydrophilic antioxidant protection, catalase, and superoxide dismutase levels in HSF/ HSF + *γ*- ORY group	
			Plasma adiponectin levels	↓	
			Protein expression	*γ*- ORY ↑ expression of Adipo-R2 and PPAR-α	
16.	Wound healing				Aldalaen *et al*. [20]
			Re-epithelization	Early epithelization by day 7	
			Histopathology	Fibroblasts, slight neo-angiogenesis, new capillaries and collagen formation on 5th day	
			Wound diameter	Rapid onset 50% reduction of wound diameters was between 7 and 10 days Complete closure of the wound was achieved after 14 days	

Table 1.
Schematic representation of therapeutic potential of γ- oryzanol.

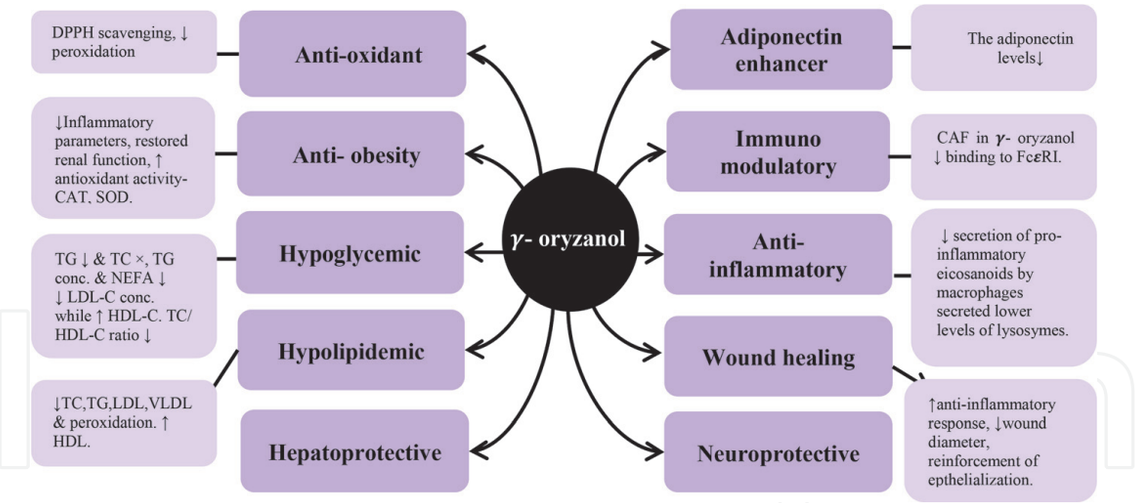

Figure 2.
Effect of γ- oryzanol on diseases.

Gamma oryzanol was unable to react with hydroxyl radicals it was therefore unable to interrupt the reaction with pnitrosodimethylaniline (PNDA). The rate of reduction of nitroblue tetrazolium (NBT) was unaffected by γ− oryzanol addition. It revealed that in the experimental conditions the compound was unable to scavenge superoxide radicals. Although weaker than alpha tocopherol, γ - oryzanol demonstrated dose dependent DPPH scavenging activity. Whether γ- oryzanol was pre-existing in the suspension or added exogenously as ethanolic solution, it was ineffective in inhibiting lipid peroxidation. In the light of the above findings, it was confirmed that γ-oryzanol is unable to compartmentalize into liposomes. During the evaluation avocado and castor oils were found to be resistant to heat induced lipid peroxidation. Particularly, castor oil proved to be impossible to evaluate in terms of antioxidant activity. In contrast rosa mosqueta oil and grape seed oil were very sensitive to oxidation. The remaining oils displayed average sensitivity to lipid peroxidation. An increase in γ- oryzanol concentration showed an increase in AI values of all the samples. AI is the antioxidative index calculated by ITs induction period of oil with the addition of antioxidant and ITo induction period of oil alone.

Free radical scavenging action of γ- oryzanol as well as its preventative nature against lipoperoxidation offer it as a viable contender for natural use as an antioxidant. γ- oryzanol offers a dose-dependent increase in induction time (of maximum capacity) while simultaneously lending protection from lipid peroxidation brought about by means of heat and O_2 exposition. This particular trait was most notable in cases of oils rich with polyunsaturated fatty acids (Rosa mosqueta, linoleic acid, grape seed oil).

Although its individual use as an antioxidant proved to be unimpressive, the use of another natural antioxidant along with γ- oryzanol can lead to enhancement of antioxidant property. The array of benefits offered in terms of pharmaceutical, cosmetic and food use "of rice bran oil which is rich in Gamma-oryzanol" suggest that γ- oryzanol can be studied further as an antioxidant component in complex lipophilic formulations such as ointments/emulsions or as an excipient for topical use.

3.2 Anti-hypercholesteremic activity

Multiple studies conducted among human beings and animals have shown that oils which constitute saturated fatty acids raise serum total cholesterol (TC) levels as well as low density lipoprotein levels. Wilson *et al*. conducted a study in Golden

Syrian Hamsters by feeding a non-purified hypercholesterolemia diet which comprised of 10% coconut oil and 0.1% cholesterol for a duration of 2 weeks, and separated into 4 groups of 12 in accordance to plasma cholesterol concentrations [7]. Blood samples were withdrawn at the 2 and 8 week marks from food deprived hamsters. At the 10 week mark (time of sacrifice) the aortic tissue was collected by administration of anesthesia. The fecal samples, were obtained towards the last 3 days of their exposure. Following procurement, the fecal samples were freeze dried and grinded prior to observation/analysis.

All the hamsters survived the complete course of the experiment. Hamsters that were fed rice bran oil (RBO) γ - oryzanol diets and hamsters that were fed coconut oil, ferulic acid diets displayed no discernable difference in terms of plasma triglyceride (TG) plasma γ tocopherol and alpha tocopherol concentrations. Although insignificant, hamsters administered RBO diet exhibited higher plasma lipid hydroperoxides (LPH) concentrations in contrast to hamsters administered ferulic acid and γ - oryzanol diets. In spite of having increased vitamin E concentrations and laminating high levels of cholesterol from their body through feces, the Coconut oil fed hamsters were found to have higher levels of aortic TC and free cholesterol in contrast to hamsters fed RBO, Ferulic acid and γ- oryzanol diets. The ratio of aortic free cholesterol to the ratio of esters were higher in RBO fed hamsters in contrast to hamsters fed the coconut oil and ferulic acid diets. RBOs contain multiple components, first being plant sterols and γ- oryzanol (unsaponifiable component) which contribute greatly to cholesterol lowering. The other component of RBOs i.e., tocotrienols assist in inhibition of cholesterol synthesis.

It was observed that both γ- oryzanol and ferulic acid both in concentrations of 0.5% each, lower plasma total cholesterol and non-high-density lipoprotein cholesterol (HDL-C) when compared to control hamsters. Increased excretion of cholesterol and its metabolic products could be the major mechanism utilized by RBOs in lowering of blood cholesterol levels. Although hamsters fed RBO, γ- oryzanol and ferulic acid visibly lowered cholesterol accumulation, of the 3, RBO and γ- oryzanol displayed a more significant decrease in ester accumulation in comparison with control. The experiment and its observations imply that at uniform dietary levels γ-oryzanol has better impact on both increase of plasma HDL-C and decrease of plasma non HDL-C in when compared to ferulic acid.

3.3 Anti-diabetic activity

An individual with type 2 Diabetes mellitus is likely to experience an increased rate of mortality as the result of cardiovascular diseases. Studies conducted at random within controlled environment have suggested that lipid lowering substances significantly reduce risk of cardiovascular diseases.

γ- oryzanol induces hypolipidemic action as well as influences reduction of aortic fatty streak formation. Palm oil was observed to significantly reduce plasma cholesterol as well as trigger growth of aortic cholesterol in relation to coconut oil within hamsters. Palm oil also tends to reduce serum lipids within healthy individuals as well as oxidative stress in rats.

The study conducted by Cheng *et al.* evaluated the impact of an effective component in RBO and $\gamma-$ oryzanol on insulin resistance and lipid metabolism within rats induced with type 2 Diabetes and treated with palm oil [8]. Diabetes was induced in Wistar rats by means of intraperitoneal injection consisting of streptozotocin, 15 minutes followed by another injection of nicotinamide. The rats are divided in three groups of 8, first group being the control, the second, Palm oil group (PO) and third group was treated with Palm Oil and γ- oryzanol (POO). After administration of diet for 5 weeks the diabetic rats were withheld from

consumption of food overnight (12 hours) and anesthetized by ether. The rats were then sacrificed by exsanguination from abdominal aorta. The plasma was then isolated by means of collection and centrifugation of blood. Plasma glucose level, triglycerides, HDL-C, LDL-C, non-esterified fatty acid (NEFA) concentration were evaluated by spectrophotometric means.

The diet had no impact on weight gain and neither did it display any side effects (diarrhea/death). No rats were dead as a result of T2DM induced by means of injection. The LDL-C concentration increased in PO groups instead of control. LDL-C in POO groups decreased when compared to PO groups. HDL-C increased in POO groups more than that of PO groups. Total cholesterol TC/HDL-C ratio was lower in POO groups than the other groups. Triglyceride concentrations were observed to increase in PO groups whereas TG concentrations decreased in POO group compared to PO group. At the end of week 5, fecal neutral sterol and bile acid content was notably higher in POO groups in contrast to control and PO groups.

The results gathered imply that PO could impair lipid metabolism in T2DM rats and γ - oryzanol a predominant component of RBO stabilizes irregular lipid status. Animals treated with $\gamma-$ oryzanol also displayed a 25% reduction in cholesterol absorption in comparison to control group. Secretion of acid and neutral sterols was notably increased in RBO administered animals. The AUC value of insulin in POO group observed prominent reduction compared to PO group. The result dictates that γ - oryzanol has tendency to increase sensitivity towards insulin in T2DM rats.

Increase of TG in plasma and liver increases output of glucose while decreasing clearance of insulin thereby promoting gluconeogenesis ultimately resulting in hyperinsulinemia and insulin resistance. Hypotriglyceridemic effect of γ- oryzanol positively impacted insulin resistance in T2DM rats. In summary, the plasma LDL-C, TG and hepatic TG all showed a decrease in concentration. The AUCs for glucose and insulin decreased in minimal concentrations within rats. Addition of γ-oryzanol to PO group minimized the negative impact of PO on lipid metabolism within T2DM rats.

3.4 Effect on male gonads

Testicular degeneration is a condition prevalent in males of domestic species; it is characterized by reduced fertility as a result of many animals being withheld in unfavorable atmospheric conditions. An increase in temperature levels promotes testicular cellular metabolism which in turn is not met with an increase in oxygen levels, thereby resulting in tissue hypoxia.

Escobar *et al.* conducted an experiment consisting of 8 rams with an average age of 10 months and weight of 35 kg bound in a surrounding with a mean temperature of 26°*C* to attain insulation [9]. The animals were administered a 10% solution of γ-oryzanol within soybean oil. The animals were divided into two groups. The first being control group that was only administered soybean oil (33 mg/ body weight) orally per day for a month. The second or the test group was administered 10% solution of γ - oryzanol in soy bean oil orally. Semen samples were collected weekly by an electroejaculator for 11 weeks and analyzed.

In case of testicular consistency and plasma levels of testosterone, there was no apparent difference between the two treatment groups. After the completion of experimental phase the animals were orchidectomized and samples were utilized to evaluate oxidative stress. The test group was observed to have a significant decline in reactive oxygen species (ROS) levels within their testes (by about 26%) when compared to the control group. In general, between week 5 and week 11, more defects were identified within the sperm of the test group as opposed to the control

group. In case of sperm motility, the largest difference was observed in week 1, with the test group displaying increased motility.

It was observed that during week 2, the test group displayed a decrease in lipid peroxidation (TBARS) levels whereas control group displayed an increase. Simultaneously there was a decrease in total anti-oxidant potential (FRAP) levels in the control group. The group receiving *γ* - oryzanol experienced a decrease in TRAP levels and an increase in the ROS levels in weeks 3 and 9. During weeks 10 and 11, there was an increase in FRAP and TBARS levels respectively in both groups.

The study did help in making the effects of heat stress on the testes and semen of the rams evident as well as reported changes that occurred throughout the duration of the experiment. Though partial protection within oxidative parameters of semen and testes were achieved by administration of *γ*- oryzanol, the experiment did not assist in improving the negative impact of heat stress among the other parameters. In fact, the administration of *γ*- oryzanol resulted in an increase in morphological abnormalities in ram on the whole.

3.5 Hepatoprotective activity

3.5.1 Acetaminophen induced hepatic injury

Liver injury because of drug abuse is termed as hepatotoxicity. Acetaminophen (APAP) which is used as an anti-pyretic as well as an analgesic when overdosed can cause acute liver injury, furthermore can lead to liver failure. Natural compounds extracted from food substances such as rice bran oil used as a source of *γ*- oryzanol are utilized for treatment of autonomic dysfunction and menopause syndrome. *γ* - oryzanol is shown to have modulatory effects on metabolic syndrome, while inhibiting oxidative stress and delaying cell aging (senescence).

Shu *et al.* performed experiment in male Kunming mice, aged 6-8 weeks. For assessment of hepatoprotective activity, 40 mice divided into 4 groups of 10 each [21]. First group served as normal, while the second received 300 mg/kg of APAP intraperitoneally, the third group was administered the same dose of APAP combined with 7 mg/kg*γ* -Oryzanol orally daily for a week, lastly the fourth group was administered the same dose of APAP with twice the dose of *γ* -oryzanol given in the third group.

γ- Oryzanol showed an undetectable cytotoxic effect on HL-7702. The viability of HL-7702 cells was decreased by APAP. Oryzanol was able to inhibit activation of Caspase-3 by APAP which leads to cell apoptosis. The intracellular accumulation of ROS plays an important role in APAP hepatotoxicity. Oryzanol decreased ROS levels in HL-7702 cells and indicated that oryzanol is capable of reversing APAP induced hepatotoxicity. Nrf2 is a crucial part of signaling pathway in anti-oxidative effect. Oryzanol aided the nuclear translocation of Nrf2, increased mRNA levels and downstream protein levels of Nrf2 like H0-1, NQ01, GCL and GCLM. Key upstream signals AMPK and GSK3B regulate Nrf2 activity, oryzanol upregulated the phosphorylation of both AMPK and GSK3B.

AMPK phosphorylation is one of the essential preceding steps in the nuclear translocation of Nrf2 and AMPK depends on phosphorylation of its substrate GSK3B. To confirm the action through AMPK/GSK3B, the test drug was challenged with the inhibitor of AMPK by compound Compund C (CC). It was observed that CC revoked oryzanol mediated phosphorylation of GSK3B eventually, obstructing the transcription of Nrf2 responsive gene. As a net effect, CC abolished the protective effect of oryzanol in APAP model. This established the fact that activation of AMPK accounts for oryzanol mediated upregulation of Nrf2 in its hepatoprotective action.

Histoarchitecture of liver remained unchanged after treatment with γ- oryzanol. AMPK/GSK3B/Nrf2 cascade can be activated by γ- oryzanol without hepatotoxicity. The liver index and serum levels of ALT, AST and LDH increased due to APAP treatment. γ- Oryzanol was able to reduce these parameters on pretreatment. APAP led to loss of hepatocyte architecture, intra-tissue hemorrhage and infiltration of inflammatory cells which were prevented by γ- oryzanol.

The number of apoptotic cells in liver increased when exposed to APAP in TUNEL and Hoechst 33258 staining assay and these were reversed by γ- oryzanol preadministration. The paracetamol intoxication increased hepatic activities of Caspase −3, −8 and − 9. A dose dependent decrease in caspases was observed with the treatment of γ - oryzanol in mice liver of APAP. Bcl-2 is an anti-apoptotic protein while Bax is a pro-apoptotic protein. Acetaminophen treatment leads to upregulation of Bax levels and downregulation of Bcl-2 levels. The effect on Bax and Bcl-2 levels was inverted by γ - oryzanol.

Exposure of liver to APAP led to increase in MDA and decrease of GSH, total superoxide dismutase (T-SOD), and total antioxidant capacity (T-AOC). These were enhanced by γ- oryzanol. Intrahepatic inflammation is a significant part of hepatotoxicity of APAP. Intrahepatic inflammatory contents- TNF-α, IL-1β, IL-6, and NO significantly increased by APAP. The inflammatory markers were restricted by γ - oryzanol. Acetaminophen increased nuclear translocation of p65 of NFκB in the liver. COX-2 and iNOS levels increased after paracetamol intoxication which in turn were suppressed by γ - oryzanol.

3.5.2 Ethanol induced liver toxicity

Ethanol consumption leads to liver injury by inducing hepatotoxicity, oxidative stress and a decrease in antioxidant levels. A therapeutic approach for treating ethanol induced hepatotoxicity is fairly sought after since the liver is among the most essential organs for metabolism of chemical compounds to obtain energy, as well as for detoxification. Trans-ferulic acid and γ - oryzanol exhibit certain physiological activities such as inhibition of tumor promotion, reduction of serum cholesterol levels, as well as antioxidant properties in several models.

Chotimarkorn and Ushio conducted a study to evaluate the effect of γ- oryzanol on ethanol induced liver injury in male C57BL mice. The investigation was carried out by administering γ- oryzanol in ethanol at the dose of 5.0 g/kg, p.o. for 30 days [11]. The experiment consisted of six groups, each group containing 15 mice. Group 1 served as a normal control and received distilled water (5.0 g/kg); group 2, negative control received ethanol (5.0 g/kg); test groups 3 and 4 were treated with trans-ferulic acid and γ- oryzanol respectively at the concentration of 0.025 mmol with ethanol (5.0 g/kg). The positive control groups 5 and 6 received trans-ferulic acid and γ- oryzanol respectively at the dose of 0.025 mmol alone. At the end of the treatment period animals were sacrificed, livers were removed and homogenated for the estimation of AST, ALT, GSH, protein, SOD, TBARS and lipid hydroperoxide by fluorescent imaging. Coadministration of trans-ferulic acid or γ- oryzanol with ethanol exhibited potent inhibition of ethanol stimulated lipid peroxidation or oxidative stress in liver. High increase in 3- PeDPPO in ethanol treated C57BL mice liver reflected high levels of lipid peroxidation. Low intensities of 3-PeDPPO was observed in γ - oryzanol treated group indicating low levels of lipid peroxidation. A significant decrease in lipid peroxide level in hepatic tissue of ferulic acid or γ- oryzanol treated mice was observed. Similarly, a significant decrease in TBARS level was seen. This demonstrated antioxidant effect of γ- oryzanol. However, the mechanism is unclear.

Gamma oryzanol or trans-ferulic acid maintain GSH levels. The co-administration significantly rose levels of GSH and SOD activity. A similar increase in SOD activity in macrophage cell line RAW 264.7 cells is reported [22]. Abnormally high level of serum aspartate and alanine transaminases in ethanol treated mice was reduced by trans-ferulic acid and *γ*- oryzanol. In the earlier studies, *γ*-oryzanol has exhibited antioxidant properties in *in-vitro* model systems namely – in cholesterol oxidation by 2,2′-azobis 2-methylpropionamidine, porcine retinal homogenate oxidation accelerated by ferric ion, pyrogallol autooxidation and pharmaceutical oils [12, 23–25]. In short, *γ*- oryzanol showed high hepatoprotective effect by preserving the livers from chemically induced injury.

Administration of daily dose of ethanol to mice resulted in visible increase in serum enzymes AST and ALT with reference to normal control, trans-ferulic acid, *γ* - oryzanol, co-administration of trans ferulic acid and *γ* - oryzanol with ethanol for 30 days. Co-administration of Trans-ferulic acid/*γ* - oryzanol to mice with ethanol for 30 days showed potent inhibition of ethanol stimulated lipid peroxidation and oxidative stress in the liver.

Trans-ferulic acid and *γ* - oryzanol reduced AST and ALT activities of ethanol. The observed significant decrease in the activity of these enzymes suggests that trans-ferulic acid and *γ* - oryzanol protects against liver injury resulting from the toxic effect of daily dose of ethanol. Furthermore, Trans-ferulic acid and *γ* - oryzanol treatment improved the antioxidative response of the liver defense system. Mechanisms for activation or induction of SOD were investigated. The study demonstrated that oral administration of trans-ferulic and *γ* - oryzanol exerted a protective action on liver injury induced by chronic ethanol ingestion.

3.5.3 CCl_4 induced liver damage

An organism when exposed to chemical agents or undergoes an infection initiates a process of hepatic fibrosis which ultimately leads to chronic liver damage. Carbon tetrachloride is utilized as a hepatotoxin to induce hepatic fibrosis in rodents. Gomes *et al*. conducted a study in adult male Swiss mice [12]. Twenty-four hours after administration of CCl_4 animals were anesthetized by sodium phenobarbital. Blood was collected and assessed for markers of hepatic damage. Liver sections were then numerically graded to assess histological features for degree of acute hepatic injury. Enzyme activities and bilirubin levels in plasma were measured. Caspase 3 and Caspase 9 activities were measured using a Caspase Glo- assay kit. Bradford method was employed to measure protein concentration using Bovine serum albumin as a standard. The data of experimental and control groups were compared.

Liver tissue from mice exposed to CCl_4 compared to the control group revealed extensive injury, vascular congestion and hepatic fibrosis. *γ*- oryzanol supplementation reduced hepatic fibrosis and reduced degree of liver damage (injuries). No histological changes were noted. Results revealed that CCl_4 exposure increased liver peroxidation. Two-way ANOVA of caspase 3 and caspase 9 revealed a significant interaction with the treatment of *γ* - oryzanol and silymarin.

Hepatic fibrosis induced by means of CCl_4 is brought about by oxidative, inflammatory and apoptopoic alterations. *γ*- oryzanol supplementation led to intermittent suppression of pathological alterations in the model of hepatic fibrosis. Damage caused in the liver by CCl_4 was principally shown as hepatic fibrosis brought about by single administration. The findings indicated that *γ*- oryzanol prevented hepatotoxicity and consequently protected against CCl_4 induced hepatic fibrosis. Lipid peroxidation is one of the primary causes of CCl_4 induced hepatic fibrosis. The findings reinforced antioxidant potential of *γ*- oryzanol

supplementation. It is implied that one of the mechanisms involved in the hepatoprotective effect of γ- oryzanol is the regulation of oxidative stress. Anti-inflammatory compounds have the potential to serve as therapeutic agents for various diseases, including, but not limited to, hepatic fibrosis. In the study conducted, it was observed that hepatic inflammatory reactions induced by CCl_4 could be suppressed by γ- oryzanol. The study demonstrated that CCl_4 administration produced a worsening effect by caspases 3 and 9 in the liver of mice, indicating that the apoptopic process had intensified. Several studies have shown that hepatic fibrosis induced by CCl_4 can lead to apoptopic pathway *in-vitro* and *in-vivo* [26, 27].

The author suggested that γ - oryzanol supplementation prevents CCl_4 induced hepatic fibrosis by modulating activity of caspases and that antioxidant action of γ-oryzanol may be at least partially associated with normalization of apoptopic process. Study demonstrates that γ- oryzanol supplementation was able to prevent CCl_4 induced hepatic fibrosis in mice by preventing oxidative, inflammatory and apoptopoic modifications.

The hepatoprotective role of γ- oryzanol in various models of liver injury are shown in **Figure 3**.

3.6 Effect on hypoadiponectinemia

The decrease in plasma adiponectin level is integrally involved in the development of insulin resistance and the resulting type 2 diabetes. In adipocyte and myotube model via nuclear factor kB (NF-kB) transcription factor pathways, palmitate, a major fatty acid observed in meat fat such as beef tallow, was known to cause insulin resistance [10].

In a study conducted by Nagasaka *et al.* (2011), serum adiponectin levels in mice were reduced from 48 to 96 h by oral administration of Beef Tallow and palmitate to approximately half the initial levels. Then γ-oryzanol, a major bioactive ingredient in rice bran, was administered and its effects on hypoadiponectinemia were examined [13].

Single dose of 0.5 ml beef tallow (beef tallow group, n = 5), 0.5 ml maize oil (control group, n = 5) or palmitate (0.17 mg/ml maize oil, n = 5) were administered orally to C57BL6j male mice aged 7 weeks and weighing between 18 and 21 g. Separately, 0.5 ml of beef tallow and maize oil containing 0.025 mmol oryzanol were also given. Blood samples were obtained from the caudal vein from 0 to 120 h per 24 h after administration. In order to estimate the total amount of adiponectin monomer secreted from Adipocyte, serum adiponectin levels were estimated. The

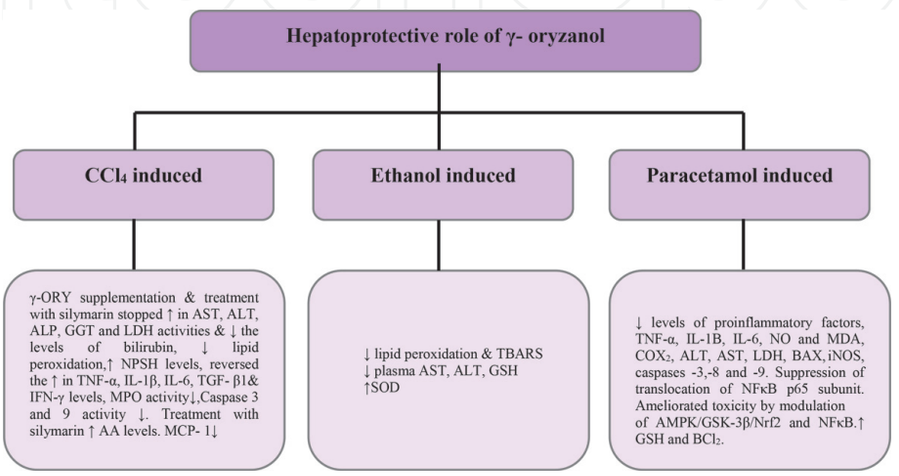

Figure 3.
Hepatoprotective role of γ- oryzanol in various models of liver injury.

immunoreactivity was observed and digitally acquired using an Odyssey Infrared imaging system. Signal intensity for adiponectin was evaluated by Image-J.

In the beef tallow group, the level of adiponectin was significantly suppressed from 48 to 72 h similarly palmitate significantly suppressed serum adiponectin levels from 48 to 96 h after administration. The administration of oryzanol dissolved in corn oil increased adiponectin level from 48 to 72 h gradually and then got the level back almost to the initial level at 120 h. Oryzanol supplementation to beef tallow increased significantly adiponectin levels at 96 h compared to the control group and successfully recovered the hypoadiponectinemia induced by the beef tallow administration. Adiponectin levels at 120 h reverted almost to the initial level, suggesting that effects of the single doses of beef tallow and oryzanol should disappear within 120 h probably due to metabolism or secretion. The rise in the secretion of adiponectin is considered a priority for the development of drugs and the treatment of metabolic diseases associated with obesity.

3.7 Impact on immune system

Fujimoto *et al.* conducted a study on cycloartenyl ferulate (CAF), a major component of rice bran derived γ -oryzanol as an anti-allergic agent in passive cutaneous anaphylaxis reaction and mast cell degranulation and its effect on IgE [14]. Gamma oryzanol was extracted from domestic Japanese rice using flash chromatography on silica gel. Passive cutaneous anaphylaxis (PCA) reaction carried out as the allergic model in Sprague Dawley rats was used in the study. Mast cell degranulation was estimated by the release of β- hexosaminidase. Light and heavy chains of anti-DNP or anti-TNP were identified by using SDS polyacrylamide gel electrophoresis. The concentrations of the compounds are given in **Table 2**.

The major component of *γ*- oryzanol was discovered to be CAF, having >90% compounds affiliated to CAF. Anti-allergic reaction of *γ*- oryzanol and CAF were found to be alike. The effect of CAF on RBL-2H3 mast cell degranulation was studied to verify that the effect of CAF on PCA reaction. Following DNP-HSA stimulation, anti-DNP IgE sensitized RBL-2H3 cells generated *β*- hexosaminidase. Anti-DNP IgE incubated with CAF was added to RBL-2H3 cells. The degranulation triggered by successive stimulation of DNP-HSA was inhibited in a concentration-dependent manner by CAF. The effect of 24-methylene cycloartanyl ferulate, cyclobranyl ferulate, and *β*- sitosteryl ferulate on mast cell degranulation on affiliation to CAF were studied by purifying the compounds. The results showed that, Cyclobranyl ferulate was more potent that cycloartanyl ferulate in inhibiting degranulation while 24 – methylene cycloartanyl ferulate and *β*-sitosteryl ferulate were found to be less effective.

It was found that CAF significantly inhibited mast cell degranulation. Binding of IgE to mast cells led to the failure of CAF to inhibit the degranulation. The researchers also found that CAF failed to inhibit degranulation once IgE binds

Compound	Concentrations
Cycloartenyl ferulate	28.2%
24 – methylene cycloartanyl ferulate	22.4%
Campesteryl ferulate	17.8%
β- sitosteryl ferulate	12.3%
Cyclobranyl ferulate	<1%

Table 2.
The predominant ferulates present in γ- oryzanol.

to mast cells. This is suggestive of some effect of CAF on the ability of IgE bind with FcϵRI.

The concentration of IgE were measured by ELISA. Anti- TNP IgE on incubation for an hour with γ- oryzanol or CAF decreased IgE concentration in a dose dependent manner. The effect was also found to be dependent on incubation time. It was also observed that ELISA failed to detect IgE when incubated with CAF.

To confirm whether CAF acted by sequestration of IgE from anti-IgE antibody or IgE configuration change SDS-PAGE analysis was performed. The amount of IgE in the supernatants decreased when IgE was incubated with CAF suggesting CAF's sequestering role on IgE which makes it undetectable in ELISA. The study demonstrated that CAF found in γ - oryzanol encapsulates IgE and prevents it from binding to FcεRI. Thereby, attenuating allergic reaction. The report of Nagasaka et al. supports the immune response of CAF as they found this molecule inhibits NFκb activity preventing the late delayed phase of allergic inflammation [22].

3.8 Anti-inflammatory activity

Serum lipid levels and pro-inflammatory mediators which are prime factors for cardiovascular diseases are greatly influenced by dietary oils. The study of Rao *et al.* investigated the effect of minor constituent of rice bran oil (RBO), γ- oryzanol on secretion of pro-inflammatory mediators by peritoneal macrophages of male Wistar rats [28]. 2 mL of fresh medium Roswell Park Memorial Institute (RPMI)-1640 was added to the macrophages and incubated with LPS. ELISA was used to study the cytokines.

The macrophages from the rats that were fed a diet with rice bran oil with unsaponifiable fraction (RBO-N) gave rise to lower levels of superoxide anion (51%) and nitric oxide (45%) compared to groundnut oil containing unsaponifiable fraction (GNO-N). However, the macrophages from rats fed rice bran oil with minor constituents removed (RBO –MCR) exhibited lower levels of superoxide anion (16%) and nitric oxide (8%) compared to GNO-N fed rats. This suggested that the extraction of unsaponifiable fraction from RBO had compromised potential to reduce the production of reactive oxygen species (ROS) by macrophages.

Lower levels of Prostaglandin E_2 (PGE_2), Thromboxane B_2 (TXB_2), Leukotriene B_4 (LTB_4) and Leukotriene C_4 (LTC_4), were secreted by macrophages from rats that were fed RBO-N diet compared to GNO-N diet fed rats. RBO-MCR diet fed group secreted lower levels of PGE_2 compared to macrophages from rats fed GNO-N. The secretion of TXB_2, LTB_4 and LTC_4 in RBO-MCR and GNO-N or GNO-MCR diet fed rats showed no remarkable differences. This deduced that the removal of unsaponifiables from RBO-N impacted its potential to effect the eicosanoid secretion by macrophages. On the contrary, rats fed RBO-N diet showed an enhanced secretion of 6-keto PGF_{1a} by 36% compared to rats given GNO-N diet.

A decrease in levels of pro-inflammatory cytokines like TNF-α (by 65%) and IL-6 (by 40%) was observed in the macrophages of rats fed with RBO diet in contrast to rats fed with GNO diet. TNF- α and IL-6 were secreted in lower levels by macrophages from rats that were fed an unsaponifiable removed RBO diet compared to rats fed with GNO diet. Pro-inflammatory response in hosts was also influenced by lysozyme enzymes secreted by macrophages. Lower levels of collagenase, elastase and hyaluronidase by 42%, 43% and 55% respectively were secreted by macrophages of rats fed with RBO diet compared to rats fed with GNO diet. GNO-MCR diet fed rats secreted collagenase, elastase and hyaluronidase in similar levels compared to GNO diet fed rats.

Pro-inflammatory compounds were secreted in lower levels by macrophages of rats fed RBO diet as compared to that observed from rats fed GNO. Reactive oxygen

species, lysosomal enzymes, eicosanoids, cytokines and matrix metalloproteases are over produced by macrophages when activation of NF-kB induces the pro-inflammatory signaling pathway [29]. The authors demonstrated how macrophages in rats fed RBO containing *γ*- oryzanol secrete less IL-1*β* in contrast to rats fed hydrogenated fat [30]. Expression of adiponectins was up regulated by RBO and down regulated expression of Toll like receptors (TLR-2 and TLR-4). The secretion of inflammatory compounds was lowered suggesting that the removal of unsaponifiables from RBO led to a decrease in the potential of RBO.

3.9 Neuroprotective role

The various neuropharmacological actions of *γ*-oryzanol are represented in **Figure 4.**

3.9.1 Anti-parkinsonian activity

Gamma oryzanol has shown potential in reduction of tumor growth and plasma cholesterol levels [31]. Ferulic acid a constituent of *γ*-oryzanol has protective function towards Alzheimer's, Parkinson's disease and stroke [32]. Parkinson's disease can be induced in Drosophila and rodents models using a chemical, rotenone [33]. Rotenoids consists of a toxic agent called rotenone [34]. Rotenone acts as an inhibitor with high affinity towards mitochondrial NADH dehydrogenase (complex I) [35]. It is suggested that dopaminergic cell death is caused by rotenone. It also causes increase in free radicals and oxidative stress in mitochondria [36].

Drosophila melanogaster is used as a genetic tool for studying biological problems because of its similarities with mammals. They have similar biological, physical and neurological properties and 75% of human disease-causing genes [37]. Several studies on neurodegenerative diseases used Drosophila as a model as it shares genetic similarity with humans in related to Parkinson's disease [38].

Araujo *et al*. performed a study using both genders of *Drosophila melanogaster* of age 1 to 5 days by dividing them into four groups of 50 flies each [16]. Control, oryzanol 25 *μ*M, rotenone 500 *μ*M, and oryzanol 25 *μ*M + rotenone 500 *μ*M. The groups were administered a diet containing rotenone and oryzanol for 7 days. Ethanol and sucrose were used as diluents with rotenone (500 *μ*M) and oryzanol (25 *μ*M) respectively. The dose of oryzanol was decided after conducting an experiment using different doses of oryzanol. Doses of 25 *μ*M, 50 *μ*M and 75 *μ*M were

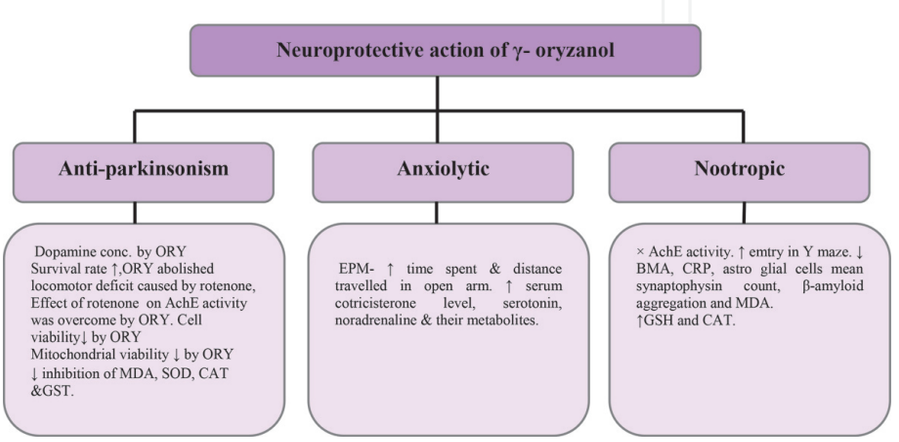

Figure 4.
Neuroprotective action of γ- oryzanol.

checked. After studying mortality and behavioral test negative geotaxia of flies, 25 μM concentration was deemed best for the experiment.

The flies were administered rotenone (500 μM) and oryzanol (25 μM). Two controls were taken one with 1% ethanol and 1% sucrose. Results presented control group with ethanol and sucrose because there was no statistical difference observed in all groups. The diet contained 1% agar w/v, 1% w/w milk powder, 0.08% w/v nipagin, 2% w/v sucrose and 1% yeast w/v beer. During the experimental period, the survival rate of the flies was estimated daily. The time taken to achieve 8 cm height from the base of the glass tube was measured to determine negative geotaxis [39]. Negative geotaxis behavior assay was used to evaluate locomotor activity of the flies [40]. Ice was used to induce anesthesia to 10 flies of both genders. The flies that were unable to climb above the mark were noted. The activity and movement of the fly were estimated by dividing 15 flies with one square cm distance in a covered petri dish [41].

A homogenate of flies was prepared which was later subjected to HPLC to analyze dopamine concentration. The cell viability was estimated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay and the resazurin reduction assay. ELISA (enzyme linked immunosorbent assay) was used for incubation of supernatant and fluorescence was noted. The flies were centrifuged to obtain mitochondria. Spectrophotometry was used with Ellmann reagent to evaluate Thiol protein and non-protein content [42]. Followed by evaluation of reactive oxygen species (ROS) and lipid peroxidation. Catalase activity was determined using method of Aebi [43]. Inhibition of quercetin auto oxidation was used to find the superoxide dismutase activity [44]. 1-chloro-2-4- dinitrobenzene (CDNB) was used according to Habig et al. procedure for the estimation of glutathione-s-transferase activity (GST) [45]. Bradford method was used to estimate protein concentration [46].

The mortality was lower in the group that was administered oryzanol, in contrast to the rotenone group. Hence, suggesting its potential to prevent rotenone induced mortality. An adverse effect was observed on the locomotor behavior on flies given rotenone, which was a decrease in climbing rate compared to the control. This was overcome by the treatment of oryzanol. In the open field test, there was a decrease in exploratory activity compared to control group, preventing a locomotor deficit. Dopamine levels in the head dropped by 42% in flies in the rotenone group compared to control group. Oryzanol prevented the drop in dopamine levels induced by rotenone in flies. Group exposed to rotenone showed MTT reduction and in cell viability observed in the fly homogenates, verifying the reduction in cell viability. Rotenone also led to a decrease in MTT reduction in mitochondria. Oryzanol reversed both the effects.

Resazurin reduction test was used to estimate cell viability. Toxicity of rotenone cell level was verified by decrease in cell. A marker of lipid peroxidation, Malondialdehyde (MDA) was estimated. MDA levels and rotenone induced DCFDA (2′,7′-dichlorodihydrofluorescein diacetate) oxidation was reduced by simultaneous exposure to oryzanol and rotenone. Oryzanol inhibited decrease Superoxide dismutase activity (SOD), catalase (CAT) and glutathione S-transferase (GST) caused by rotenone. The amount of protein thiols and non-protein thiols did not change and remained the same in all groups.

Motor function, dopamine levels and activity of enzyme acetylcholinesterase improved with the treatment of oryzanol. In addition, oryzanol strengthened anti-oxidant defenses, oxidative stress, mitochondrial dysfunction protecting from rotenone toxicity. The constituent of oryzanol, ferulic acid esters could be responsible for the neuroprotective role and its anti-oxidant ability. Ferulic acid demonstrated anti-oxidant activity in neuronal cell culture and arrested apoptosis in focal cerebral

ischemic injury showing neuroprotective action. Ferulic acid on long term administration in mice ameliorated memory deficits induced by centrally administered $\beta-$amyloid [47]. Decrease in expression of active caspase-3 in the rat striatum, increase in interlukin-1 an immunoreactive component, levels of endothelial nitric oxide synthase and 3- nitrotyrosine of mouse hippocampus can be mediated by feruilic acid. It also provides neuroprotection against striatal neuronal cells exposed to oxidized low-density lipoproteins [48]. Neuromotor deficits, geotaxia negative tests (climbing) and open field test (rating exploratory capacity) were successfully reversed by oryzanol.

Rotenone is used to induce symptoms of Parkinson's disease. As Parkinson's disease is related to mitochondrial dysfunction because of anomaly in complex I of electron transport chain, similar symptoms can be induced using rotenone [49]. A decrease in anti-oxidant and an increase in iron levels along with oxidative stress on dopaminergic neurons could be carried out by the inhibition of complex I. Dopamine metabolism involves synthesis, storages, release, reuptake and degradation of neurotransmitter [50]. The flies that were treated with rotenone showed a decrease in dopamine levels. Earlier studies verified that dopamine levels in flies reduce upon treatment with rotenone including loss of dopaminergic neurons in brain and reduction in vesicular monoamine transporter (VMAT) [51, 52]. Oryzanol displayed a neuroprotective effect by preventing the dopamine loss. Further, ferulic acid has shown neuroprotective effect via inhibition COX-2 enzyme, which in turn prevents the oxidation of dopamine and prevents accumulation of α- synuclein [53].

Neurodegeneration in Parkinson's disease is also induced by oxidative stress [54]. The increase in MDA, ROS levels and decrease in CAT, SOD and GST during the study confirms that exposure to rotenone causes oxidative stress. Oryzanol was able to reduce oxidative markers thus, confirming its anti-oxidant ability. Biological membranes can be protected from lipid peroxidation, peroxyl and alkoxyl radicals by ferulic acid [55].

Cell viability was reduced when exposed to rotenone and led to increase in mortality of flies. However, oryzanol treatment increased cell viability and reduced mortality. This suggests the presence of bioactive compounds in it that suppress free radicals and contribute to the anti-oxidant defense system. Finally, the study concluded that oryzanol prevented the toxicities caused by rotenone in *Drosophila melanogaster*. Thereby, confirming its neuroprotective role in Parkinson Disease.

3.9.2 Anti-anxiety

It is believed that chronic stress is correlated to structural degeneration and compromised brain function which could be the reason for increased risk of advancement of neuropsychiatric disorders like anxiety, depression and dementia. In an experiment conducted by Akter *et al*. five-week-old ICR male mice were put through restraint stress by a wire mesh bag (3x6x12) [17]. The mice were subjected to 1st phase chronic restraint treatment for 14 days followed by a recovery phase and second phase for another 5 days. Since, most studies employed 0.5% dose of γ - oryzanol and found it to be effective, 0.5% γ - oryzanol was administered to the mice.

Open field and elevated plus maze tests were used as behavioral tests. Mice were sacrificed at ZT 5.5 to 7.5 post 3 h CRST exposure and EPM test. Samples were drawn and serum was separated after centrifugation for 15 min at 3000 rpm and stored at $-80°C$. Brain matrix was used for excision of hippocampus, cerebral cortex and amygdala. Neuroanatomical landmarks from the brain atlas was used to dissect the brain. ELISA was used to determine levels of serum corticosterone.

The neurochemical mechanisms involved in anxiolytic like effects were studied by estimating the levels of centrally acting monoamine neurotransmitters such as noradrenaline and serotonin and their metabolites 5-hydroxyindole acetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), in the various dissected parts of the brain by HPLC-ECD following the behavioral tests.

GORZ treatment did not show any significant changes in the behavioral parameters in both the tests. Daily food intake sharply decreased in the stressed mice group during the early days of CRST. However, there was substantial recovery in the later period. As a result of CRST negative control group significantly lost body weight when compared to the normal control and test group. The treatment of 0.5% γ- oryzanol reduced such effects indicating that the γ- oryzanol treatment prevented the stress induced weight loss.

A mild reversal of CRST induced decrease in time spent in the central zone was observed by the treatment of γ- oryzanol. 0.5% oryzanol treatment significantly increased number of entries in the central zone in CRST while slightly increasing the total distance traveled in both the conditions. These are suggestive of the decreasing effect of γ- oryzanol on anxiety like behavior. In elevated plus maze test, γ- oryzanol significantly increased time spent in open arm and distance traveled in open arm under cold restrained stress. At the same time oryzanol treatment reduced the distance traveled in the closed arm. These observations indicate the anxiolytic effect of γ- oryzanol. Serum corticosterone levels rose significantly in control and γ-oryzanol treated animals in contrast with the unstressed control animals in CRST.

A mild reduction in the levels of neurotransmitters and their metabolites following CRST was observed in the hippocampus and cerebral cortex. CRST led to a decrease in serotonin and 5-HIAA levels with no significant change in amygdala. The slight decrease in serotonin and nor-adrenaline and their metabolites was restored by oryzanol in the hippocampus and cerebral cortex. Under CRST an increase in 5-HIAA, a metabolite of 5-HT was seen in amygdala of mice that received 0.5% of oryzanol. Similarly, an increase in a noradrenaline metabolite (MHPG) was noticed in oryzanol treated group without stress. These facts are implicative of anti-anxiety potential of γ- oryzanol in chronic stress conditions. CRST induces morphological alterations in BBB. It also remarkably decreased body weight (b.w.) by inhibiting food intake through the reduction of mRNA expression of food intake related genes like ghrelin, pro-opiomelanocortin in hypothalamus. Gamma oryzanol is indicated to be a weak modulator of stress response in hippocampus. Its anxiolytic effect could be related to the up regulation of centrally acting monoamines in amygdala. The anti-stress and anti-anxiety effects are unrelated to corticosterone activity. In gist, the observations of elevated plus maze test evidence the anti-anxiety effect of oryzanol. The locomotor activity was found to be unaffected.

3.9.3 Anti-Alzheimer

The neuroprotective and cognitive enhancement effect of γ- oryzanol in Alzheimer's disease was investigated by Jha and Panchal [18]. In the study, the researchers performed *in-vitro* DPPH assay, AchE enzyme activity inhibition assay, cell viability assay on SH-SY 5Y cell line and alamar blue assay. The nootropic activity was assessed using Y and radial arm maze. The brain corticular homogenate was subjected to estimation of biochemical markers such as catalase, glutathione, malondialdehyde, brain mitochondrial ATPase, brain acetylcholinesterase activity and C-reactive protein. The slices of brain were finally subjected to amyloid-β-plaque staining, immunohistochemistry and histopathology. The IC_{50} of γ- oryzanol was found to be 227.03 ± 17.24 μM in DPPH assay and 34.04 ± 3.20 μM in AchE

inhibitory assay. γ- oryzanol has shown a dose-dependent enzyme inhibition by preventing hydrolysis of ATCI from AchE *in-vitro*. γ- oryzanol treatment increased cell survival by 1.07-1.104 folds in the cell viability assay at concentrations 100 nM, 1 μM and 100 μM. In both Y and radial arm maze tests, γ- oryzanol raised entry in Y maze, exhibited significant increase in total arm entry and correct arm entry, reduced reference as well as working memory errors. Memory score improved with the treatment of Donepezil and γ- oryzanol treated animals. γ- oryzanol increased the level of brain mitochondrial ATPase (BMA), catalase (CAT) and glutathione (GSH) while significantly decreasing the malondialdehyde (MDA), acetylcholinesterase activity and C reactive proteins (CRP) thereby exhibiting free radical scavenging action in a dose dependent manner and was found to be more potent than ferulic acid.

The histopathological examination revealed regular morphological and cytological characteristics in normal control group and disorganized cellular and morphological architecture, presence of dead cells, loss of neuronal cells in CA_1, CA_2, CA_3 and DG regions, altered thickness (CA_1 and CA_2), aberrantly scattered CA_3 pyramidal cells, cell arrangement in granular cell layer (GCL), dentate gyrus ectal limb (DGEC), dentate gyrus endal limb (DGEN) and loss of neuronal cells in entorhinal cortex (ERC) in disease control group. There were no such observations in Donepezil and γ- oryzanol treated brains. Immunohistochemical analysis showed non-significant but marked reduction in Mean GFAP count, decrease in active astroglial cells and inflammation. γ- oryzanol group brain sections improved synaptic connectivity, indicated by increase in the mean synaptophysin count in CA_1, CA_2, CA_3, DG and ERC regions. This highlighted protective effect of γ- oryzanol in streptozotocin induce cerebral damage.

The qualitative and quantitative analysis of cortical area of brains observed as bright red fluorescence revealed higher mean amyloid-β count in disease control group which was lowered by both Donepezil and γ- oryzanol as it decreased plaque formation. The immunohistochemical parameters (GFAP and synaptophysin) expression and amyloid β-12 was inhibited by γ- oryzanol. The overall effects of γ-oryzanol were suppression of neuroinflammation and plaque formation (β-amyloid aggregation) while improving synaptic connectivity and neuronal energy catastrophe in cerebral region preventing neuronal loss. γ - oryzanol proved to be beneficial and therapeutic candidate in the experimental model of sporadic Alzheimer disease and demonstrated potent anti-oxidative, anti-inflammatory, cognitive enhancing and amyloidogenesis terminating effects. The researchers recommended further studies and exploration of γ- oryzanol use in the neurodegenerative disorder.

3.10 Anti-obesity potential

As γ- oryzanol proved to have the ability to treat hyperlipidemia, hyperglycemia, hypoadiponectinemia, etc., the nutraceutical was considered to be a good candidate for screening in obesity induced kidney injury by Francisqueti *et al*. [19].

Male Wistar were divided into 2 groups, control group and high sugar-fat diet (HSF) group after 20 weeks, the rats were treated with γ- oryzanol. Post treatment, the rats caloric intake, body weight and adiposity index were used to estimate their nutritional profile. Glucose concentration, triglycerides and adiponectin were estimated using glucometer, automatic enzyme analyzer system and enzyme-linked immunosorbent assay (ELISA) respectively. Plasma and urine were used to estimate the renal function. Amount of urea and creatinine in plasma was recorded along with the glomerular filtration rate (GFR). The renal tissue was homogenized and centrifuged. ELISA was used to measure tumor necrosis factor - alpha (TNF – α),

interlukin - 6 (IL-6) and monocyte chemoattractant protein - 1 (MCP–1) levels. Protein amount was used to verify the results.

The caloric intake showed no change and HSF showed higher values for all parameters. The renal function of the group that was given HSF and γ- oryzanol presented lower proteinuria and high GFR. Renal tissue of the group that was administered γ- oryzanol showed decrease in inflammatory response unlike the control group. During the study, the group given HSF diet developed obesity, insulin resistance, hypertension, chronic inflammation, dyslipidemia and oxidative stress.

Antioxidant defense is impaired in subjects suffering from renal insufficiency. γ-oryzanol treatment exhibited a rise in antioxidant capacity - superoxide dismutase (SOD) and catalase activity. Abnormal levels of adiponectin are seen in obesity, diabetes, chronic kidney disease, etc. The secretion of adiponectin by adipose tissue has a great impact on kidney disease. In contrast to reduced levels of adiponectin when it comes to obesity, chronic kidney disease shows rise in the same. Therefore, the higher levels of adiponectin related to GFR in the HSF fed group helped to deduce kidney disease. On the contrary, the HSF diet group treated with γ- oryzanol showed reduced adiponectin levels. The expression of PPAR – α increases in tissue with high mitochondrial and $\beta-$oxidation activity. Increased PPAR – α expression suggests metabolic control in an organ, and it controls several factors involved in renal damage [56, 57]. Thus, supporting the study to conclude that HSF diet with oryzanol showed no inflammation in kidney, as well as low levels of TNF-α, IL-6, and MCP-1.

3.11 Wound healing activity

γ – Oryzanol has both anti-inflammatory and antioxidant properties, which makes it a prospect for wound healing. However, due to the large molecular weight and water insolubility, it is incompatible for topical application. Penetration enhancers containing vesicles were formulated using used transcutol and labrosol in a study conducted by Aldalaen *et al.* [20]. Alpha – bisabolol (BISA) is a derivative of essential oil. BISA is an unsaturated sesquiterpene alcohol. Reports indicate that BISA is employed as a nutraceutical compound in treating wounds. Owing to its anti-inflammatory property, BISA was included as co-penetrator to boost the permeation ability of γ - oryzanol. Thus, increasing its antioxidant and anti-inflammatory properties.

The formulation was developed using the thin film hydration technique. γ-oryzanol (25 mg) and the phospholipid Epikuron (200 mg) were dissolved in a mixture of chloroform: methanol (2:1) v/v. This mixture was then subjected to vacuum evaporation at 40°*C* and 150 rpm. The lipidic film was then hydrated with 10 ml phosphate buffer (pH 7.4) comprising of BISA oil (10 μL) and penetration enhancers (transcutol and labrosol). The vesicular dispersion was treated with rotation for 30 mins at 40°*C* and sonication for 1 hr. Finally, storing it at 4°*C*.

The experiment was conducted in Wistar rats. Intraperitoneal (i.p) ketamine HCL (50 mg/kg) and xylazine HCL (20 mg/kg) were used as anesthetics. The animals were subjected to excision wound model. The posterior of the rats were shaved and cleaned with 70% ethanol. Two circular wounds were induced on the dorsal skin of the rats. A biopsy punch of 20 mm in diameter was utilized for the removal of the skin. The Wistar rats were divided into three groups. The wound on the upper side of the dorsal back was treated while the lower side was left untreated which was used as control. The PEV formulation with γ- oryzanol was given to group 1, the PEV formulation with γ -oryzanol and BISA was given to group 2, and group 3 was treated with commercial wound healing product (Healosol). Fifty μ L of each formulation was applied on the upper side of the wounds every day. The rats were photographed, and

the diameter of wounds were measured on the 3,5,7,10,14 and 21 days. The rats were subjected to euthanasia and the tissue formed was cut out leaving 5 mm of skin. The skin samples were then examined histopathological changes.

During the experiment it was observed that the wound treated with PEV formulation including γ -oryzanol and BISA exhibited a better anti-inflammatory response and wound healing capacity. This formulation could enhance the wound healing due to the pharmacological effects of both γ -oryzanol and BISA, and their antioxidant properties. Gamma oryzanol induced morphological abnormalities in the testes of ram indicating some form of reproductive toxicity. But this necessitates careful scrutiny of the effects of γ – oryzanol on testes and ovaries.

4. Conclusion

Among the noteworthy observations of γ – oryzanol were the acetylcholinesterase inhibitory action, decrease in brain mitochondrial ATPase, increase in mean synaptophysin count, and decrease in astroglial cell in an *in-vitro* Alzheimer study. Another significant discovery was AMPK/ GSK3β/Nrf2 and NFκB modulated hepatoprotection in APAP induced liver injury. γ – oryzanol promoted nuclear translocation of Nrf-2, increasing its expression modulated AMPK/GSK3β axis, suppressed nuclear translocation of NFκB p65 subunit, down regulating expression of iNOS and COX-2. The same experiment also proved limiting action on TNF-α, IL-Iβ, IL-6, nitric oxide. Though, above mechanisms have been established there are few bioactivities where the exact mechanisms of action are yet to be confirmed. Therefore, further exhaustive research is required to unveil the mechanisms and to explore the utility of γ – oryzanol in other disease states.

From the bulk of information related to the nutritional value and diverse therapeutic potentials of gamma-oryzanol, it is concluded that this optimistic molecule can be recognized as a nutraceutical and utilized in the management of various diseases. However, the beneficial role of gamma-oryzanol in certain conditions is not fully understood, necessitating further exhaustive studies to establish the mechanism of action.

Author details

Aasiya Sulaiman[1], Aisha Sulaiman[1], Mehtap Sert[2], Mohammed Safwan Ali Khan[3,4]* and Mansoor A. Khan[5]

1 Stay Fit Fitness Club, Dubai, United Arab Emirates

2 Turkiye Saglik Enstituleri Baskanligi (Health Institutes of Turkey), Istanbul Yerleskesi, Istanbul, Turkey

3 Hamidiye International Faculty of Medicine, Department of Pharmacology, University of Health Sciences, Istanbul, Turkey

4 Department of Biomedical Sciences, School of Medicine, Nazerbayev University, Nur-Sultan, Kazakhstan

5 Department of Pharmaceutical Sciences, Irma Lerma Rangel College of Pharmacy, Texas A&M Health Science Center, Texas A&M University, Texas, USA

*Address all correspondence to: safwan.aucp@gmail.com; safwan.npr@gmail.com

References

[1] Srikaeo K. Organic Rice Bran Oils in Health. In: Wheat and Rice in Disease Prevention and Health. Elsevier; 2014. p. 453–65. doi.org/10.1016/B978-0-12-401716-0.00035-0

[2] Xu Z, Godber JS. Purification and identification of components of gamma-oryzanol in rice bran Oil. J Agric Food Chem. 1999 Jul;47(7):2724-2728. DOI: 10.1021/jf981175j

[3] Rungratanawanich W, Abate G, Uberti D. Pharmacological profile of γ-oryzanol: Its antioxidant mechanisms and its effects in age-related diseases. In: Aging. Elsevier; 2020. p. 201–208. Doi: 10.1016/B978-0-12-818698-5.00020-1.

[4] Fujiwara S, Noumi K, Sugimoto I, Awata N. Mass fragmentographic determination of ferulic acid in plasma after oral administration of Gamma-oryzanol. Chem Pharm Bull. 1982;30(3): 973–979. Doi: 10.1248/cpb.30.973.

[5] Moon S-H, Kim D, Shimizu N, Okada T, Hitoe S, Shimoda H. Ninety-day oral toxicity study of rice-derived γ-oryzanol in Sprague-Dawley rats. Toxicology Reports. 2017;4:9–18. Doi:10.1016/B978-0-12-818698-5.00020-1.

[6] Juliano C, Cossu M, Alamanni MC, Piu L. Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils. International Journal of Pharmaceutics. 2005 Aug;299 (1–2):146–54Doi:10.1016/j.ijpharm.2005.05.018

[7] Wilson TA, Nicolosi RJ, Woolfrey B, Kritchevsky D. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. The Journal of Nutritional Biochemistry. 2007 Feb;18(2):105–112.

[8] Cheng H-H, Ma C-Y, Chou T-W, Chen Y-Y, Lai M-H. Gamma-oryzanol Ameliorates Insulin Resistance and Hyperlipidemia in Rats with Streptozotocin/nicotinamide-induced Type 2 Diabetes. International Journal for Vitamin and Nutrition Research [Internet]. 2010 Jan 1;80(1):45–53. Available from: http://dx.doi.org/10.1024/0300-9831/a000005

[9] Escobar E, Lopes S, Malavolta C, Ramalho JB, Missio D, Pinto HF. Effect of γ-oryzanol on testicular degeneration induced by scrotal insulation in rams. Theriogenology. 2019 Apr;128:167–175.

[10] Chaves, J.A., Summers, S.A., 2003. Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. Arch. Biochem. Biophys. 419, 101–109.

[11] Chotimarkorn C, Ushio H. The effect of trans-ferulic acid and gamma-oryzanol on ethanol-induced liver injury in C57BL mouse. Phytomedicine. 2008 Nov;15(11):951–958. Doi: 10.1016/j.phymed.2008.02.014

[12] de Gomes MG, Del Fabbro L, Goes AR, Souza LC, Boeira SP, Haas SE, Puntel RL, Jesse CR. Supplementation with gamma oryzanol ameliorates CCl4-induced hepatic fibrosis in mice. PharmaNutrition. 2019 Dec;10:100169. Doi:10.1016/j.phanu.2019.100169

[13] Nagasaka R, Yamsaki T, Uchida A, Ohara K, Ushio H. γ-Oryzanol recovers mouse hypoadiponectinemia induced by animal fat ingestion. Phytomedicine. 2011 Jun;18(8–9):669–671. Doi:10.1016/j.phymed.2011.01.004

[14] Oka T, Fujimoto M, Nagasaka R, Ushio H, Hori M, Ozaki H. Cycloartenyl ferulate, a component of rice bran oil-derived γ-oryzanol, attenuates mast cell

degranulation. Phytomedicine. 2010 Feb;17(2):152–156. Doi:10.1016/j.phymed.2009.05.013

[15] Rao YPC, Sugasini D, Lokesh BR. Dietary gamma oryzanol plays a significant role in the anti-inflammatory activity of rice bran oil by decreasing pro-inflammatory mediators secreted by peritoneal macrophages of rats. Biochemical and Biophysical Research Communications. 2016 Oct;479(4):747–752. Doi:10.1016/j.bbrc.2016.09.140

[16] Araujo SM, de Paula MT, Poetini MR, Meichtry L, Bortolotto VC, Zarzecki MS, Jesse CR, Prigol M. Effectiveness of γ-oryzanol in reducing neuromotor deficits, dopamine depletion and oxidative stress in a Drosophila melanogaster model of Parkinson's disease induced by rotenone. NeuroToxicology. 2015 Dec;51:96–105. Doi:10.1016/j.neuro.2015.09.003

[17] Akter S, Sasaki H, Uddin KR, Ikeda Y, Miyakawa H, Shibata S. Anxiolytic effects of γ-oryzanol in chronically-stressed mice are related to monoamine levels in the brain. Life Sciences. 2019 Jan;216:119–128. Doi:10.1016/j.lfs.2018.11.042

[18] Jha AB, Panchal SS. Neuroprotection and cognitive enhancement by treatment with γ-oryzanol in sporadic Alzheimer's disease. JAB. 2017 Nov 1;15(4):265–281. Doi: 10.1016/j.jab.2017.05.001

[19] Francisqueti FV, Ferron AJT, Hasimoto FK, Alves PHR, Garcia JL, dos Santos KC, Moreto F, Silva V dos S, Ferreira ALA, Minatel IO, Corrêa CR. Gamma Oryzanol Treats Obesity-Induced Kidney Injuries by Modulating the Adiponectin Receptor 2/PPAR-α Axis. Oxidative Medicine and Cellular Longevity 2018 Sep 9;2018:1–9.Doi: 10.1155/2018/1278392

[20] Aldalaen S, Nasr M, El-Gogary RI. Angiogenesis and collagen promoting nutraceutical-loaded nanovesicles for wound healing. Journal of Drug Delivery Science and Technology. 2020 Apr;56:101548. Doi:10.1016/j.jddst.2020.101548

[21] Shu G, Qiu Y, Hao J, Fu Q, Deng X. γ-Oryzanol alleviates acetaminophen-induced liver injury: roles of modulating AMPK/GSK3β/Nrf2 and NF-κB signaling pathways. Food Funct. 2019; 10(10):6858–6872. Doi:10.1039/C9FO01808E

[22] Xu, Z., Hua, N., Godber, J.G., 2001. Antioxidant activity of tocopherols, tocotrienols, and gamma-oryzanol components from rice bran against cholesterol oxidation accelerated by 20 2-azobis (2-methypropionamidine) dihydrochloride. J. Agric. Food Chem. 49, 2077–2081.

[23] Hiramitsu, T., Armstrong, D., 1991. Preventive effects of antioxidants on lipid peroxidation in the retina. Ophthalmic Res. 23, 196–203.

[24] Kim, J.S., Godber, J.S., 2001. Oxidative stability and vitamin E levels increased in restructure beef roast with added rice bran oil. J. Food Qual. 24, 17–26.

[25] Juliano, C., Cossu, M., Alamanni, M. C., Pui, L., 2005. Antioxidative activity of gamma-oryzanol: mechanism of action and its effects on oxidative stability of pharmaceutical oil. Int. J. Pharm. 299, 146–154.

[26] F. Sun, E. Hamagawa, C. Tsutsui, Y. Ono, Y. Ogiri, S. Kojo, Evaluation of oxidative stress during apoptosis and necrosis caused by carbon tetrachloride in rat liver, Biochim. Biophys. Acta 1535 (2001) 186–191.

[27] Y. Cai, L.K. Gong, X.M. Qi, X.H. Li, J. Ren, Apoptosis initiated by carbon tetrachloride in mitochondria of rat primary cultured hepatocytes, Acta Pharmacol. Sin. 27 (2005) 969–975.

[28] Nagasaka, R., Chotimarkorn, C., Shafiqul, I.M., Hori, M., Ozaki, H., Ushio, H., 2007. Anti-inflammatory effects of hydroxycinnamic acid derivatives. Biochem. Biophys. Res. Commun. 358, 615–619.

[29] S. Sakai, T. Murata, Y. Tsubosaka, H. Ushio, M. Hori, H. Ozaki, g-Oryzanol reduces adhesion molecule expression in vascular endothelial cells via suppression of nuclear factor-kB activation, J. Agric. Food. Chem. 60 (2012) 3367e3372.

[30] Y.P.C. Rao, P. Pavan Kumar, B.R. Lokesh, Molecular mechanisms for the modulation of selected inflammatory markers by dietary rice bran oil in rats fed partially hydrogenated vegetable fat, Lipids 51 (2016) 451e467.

[31] Wang YX, et al. Hypolipidemic and Antioxidative Effects of Aqueous Enzymatic. Extract from Rice Bran in Rats Fed a High-Fat and -Cholesterol Diet. Nutrients 2014; v 6. doi:10.3390/nu609396.

[32] Cheng CY, Su SY, Tang NY, Ho TY, Chiang SY, Hsieh CL. Ferulic acid provides neuroprotection against oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury by inhibiting of ICAM-1 mRNA expression in rats. Brain Res 2008; 13, 136–150. doi: 10.1016/j.brainres.2008.02.090

[33] Tamilselvam K, Braidy N, Manivasagam T, Essa M.M, Prasad N.R, Karthikeyan S, Thenmozhi A.J, Selvaraju S, Guillemin G.J. Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a celluar model for Parkinson's disease. Oxid. Med. Cell. Longev 2013; V 2013, 11 pages. DOI: 749 10.1155/2013/102741

[34] Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F,Umbach DM, Blair A, Sandler DP, Langston JW. Rotenone, paraquat, and Parkinson's disease. Environmental health perspectives 2011;119:866–872. doi: 10.1289/ehp.1002839

[35] Cicchetti F, Drouin-Ouellet J, Gross RE. Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models?. Trends Pharmacol Sci 2009;30: 475–483. doi: 10.1016/j.tips.2009.06.005.

[36] Perfeito, R. Rego AC. Papel da alfa-sinucleína e da disfunção mitocondrial associada à doença de Parkinson. Rev Neurocienc 2012;20(2):273-284.

[37] Pandey UB, Nichols CD. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol Rev 2011; 63: 411–436. doi:10.1124/pr.110.00329321415126.

[38] Whitworth AJ, Wes DP, Pallanck LJ. Drosophila models pioneer a new approach to drug discovery for Parkinson's disease. Drug Discovery Today 2006. v. 11, n. 3-4, p.119–126. doi: 10.1016/S1359-6446(05)03693-7.

[39] Jimenez-Del-Rio M, Guzman-Martinez C, Velez-Pardo C. The Effects of Polyphenols on Survival and Locomotor Activity in Drosophila melanogaster Exposed to Iron and Paraquat. Neurochem Res. 2009 Aug 23; 35(2):227–238. Doi: 10.1007/s11064-009-0046-1

[40] Coulom H. Chronic Exposure to Rotenone Models Sporadic Parkinson's Disease in Drosophila melanogaster. Journal of Neuroscience. 2004 Dec 1;24 (48):10993–10998. Doi:10.1523/JNEUROSCI.2993-04.2004

[41] Hirth F. Drosophila melanogaster in the Study of Human Neurodegeneration. CNSNDDT. 2010

Aug 1;9(4):504–523. Doi:10.2174/187152710791556104

[42] Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959 May;82(1):70–77.

[43] Aebi H. Catalase *in-vitro*. Methods Enzymol. 1984; 105:121–126.

[44] Venkareddy LK, Muralidhara. Potential of casein as a nutrient intervention to alleviate lead (Pb) acetate-mediated oxidative stress and neurotoxicity: First evidence in Drosophila melanogaster. NeuroToxicology. 2015 May;48:142–151. Doi:10.1016/j.neuro.2015.03.014

[45] Ramesh E, Elanchezhian R, Sakthivel M, Jayakumar T, Senthil Kumar RS, Geraldine P, Thomas PA. Epigallocatechin gallate improves serum lipid profile and erythrocyte and cardiac tissue antioxidant parameters in Wistar rats fed an atherogenic diet. Fundam Clin Pharmacol. 2008 Jun;22(3):275–284.

[46] Mishra A, Patel MK, Jha B. Non-targeted metabolomics and scavenging activity of reactive oxygen species reveal the potential of Salicornia brachiata as a functional food. Journal of Functional Foods. 2015 Mar;13:21–31. Doi:10.1016/j.jff.2014.12.027

[47] Kim HS, Cho JY, Kim DH, Yan JJ, Lee HK, Suh HW, Song DK. Inhibitory effects of long term administration of ferulic acid on microgilal activation induced by intercerebroventricular injection of beta-amyloid peptide (1-42) in mice. Biol. Pharm. Bull. 2004;27:120–121. doi: 10.3164/jcbn.40.92.

[48] Schroeter H, Williams RJ, Matin R, Iversen L, Rice-Evans CA. Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. Free Radical Biol Med 2000;12, 1222–1233. doi: 10.1016/S0891-5849(00)00415-9

[49] Cicchetti F, Drouin-Ouellet J, Gross RE. Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models?. Trends Pharmacol Sci 2009;30: 475–483. doi: 10.1016/j.tips.2009.06.005.

[50] Qi Z, Miller GW, Voit EO. Computational Systems Analysis of Dopamine Metabolism. Hashimoto K, ed. PLoS ONE. 2008; 3(6):e2444. doi: 10.1371/journal.pone.0002444.

[51] Coulom H, Birman S. Chronic exposure to rotenone models sporadic Parkinson's disease in Drosophila melanogaster. J Neurosci 2004; 24: 10993–10998. doi:10.1523/JNEUROSCI.2993-04.2004

[52] Lawal HO, Chang H-Y, Terrell AN, Brooks ES, Pulido D, Simon AF, Krantz DE. The Drosophila vesicular monoamine transporter reduces pesticide-induced loss of dopaminergic neurons. Neurobiology of Disease. 2010 Oct;40(1):102–112.

[53] Gupta S, Benzeroual K. Neuroprotective effects of antioxidants, Idebenone and Ferulic Acid, in MPTP/MPP+ intoxicated PC12 cells as a model of Parkinson's Disease. The FASEB Journal 2013;27:1175.7.

[54] Muñoz-soriano V, Paricio N. Drosophila Models of Parkinson's Disease: Discovering Relevant Pathways and Novel Therapeutic Strategies. Parkinsons Dis 2011.; 2011: 520640. doi.org/10.4061/2011/520640

[55] Trombino S, Serini S, Di Nicuolo F, Celleno L, Ando S, Picci N, Calviello G, Palozza P. Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with alpha-tocopherol, beta-carotene, and ascorbic acid. J. Agric. Food Chem 2004; 52 2411–2420. doi: 10.1021/jf0303924

[56] Tanaka Y, Kume S, Araki SI, Isshiki K, Masami Chin-Kanasaki, Sakaguchi

M, Sugimoto T, Koya D, Haneda M, Kashiwagi A, Maegawa H, Uzu T "Fenofibrate, a PPARα agonist, has renoprotective effects in mice by enhancing renal lipolysis," Kidney International, vol. 79, no. 8, pp. 871–882, 2011.

[57] Kim EN, Lim JH, Kim MY, Kim HW, Park CW, Chang YS, Choi BS "PPARα agonist, fenofibrate, ameliorates age-related renal injury," Experimental Gerontology, vol. 81, pp. 42–50, 2016.