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RESEARCH ARTICLE

COMBINED EFFECT OF CARVACROL AND ROSIGLITAZONE ON ANTIOXIDANT STATUS IN HIGH FAT DIET INDUCED C57BL/6J DIABETIC MICE

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ABSTRACT

The objective of the study was to investigate the combination of carvacrol (CVL) and rosiglitazone (RSG) on non-enzymatic and enzymatic antioxidants in the plasma and tissues of high fat diet (HFD) induced diabetes in C57BL/6J mice weighing 20-30 g. The normal and diabetic mice were treated with CVL (20 mg/kg BW) dissolved in 0.5% dimethyl sulfoxide (DMSO) and RSG (4 mg/kg BW) dissolved in water for 35 days. Diabetic mice had an elevation in the levels of lipid peroxidation markers, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH) and conjugated dienes (CD) also a reduction in non-enzymatic antioxidants, vitamin C, vitamin E and reduced glutathione (GSH) in the plasma and tissues, and enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S- transferase in the erythrocyte and tissues. Treatment with CVL and RSG in combination brought the non-enzymatic and enzymatic antioxidants to near normality when compare to other groups.

INTRODUCTION

Over the past decades the incidence of obesity has been steadily increasing towards epidemic proportions (WHO. 2006). Although the impact of obesity as a disease is still not well accepted in public, the constant rise of obesity-associated comorbidities, such as T2DM, cardiovascular diseases and cancer, demonstrate the necessity to define the molecular mechanisms underlying the onset, manifestation and progression of obesity, as well as of its associated comorbidities to obesity. T2DM was characterized by chronic hyperglycemia and initially hyperinsulinemia, due to relative insulin resistance of insulin-target tissues. High fat diet induced oxidative stress depicts the existence of products called free radicals and reactive oxygen species, which are formed in normal physiology but become deleterious when not being quenched by a cascade of antioxidant systems. This can result either from an over production of reactive oxygen species or from the inactivation of the antioxidant system, thus shifting the oxidative stress/antioxidant system balance in favour of stress (Hayden and Tyagi 2002, Fang *et al*. 2002). ROS oxidize various types of biomolecules, finally leading to cellular lesions by damaging DNA or stimulating apoptosis for cell death. Some ROS were considered more important than others, such as superoxide, hydroxyl radicals or peroxides. ROS were neutralized by a battery of active oxygen species (AOS), which can be divided into mainly two categories: enzymatic (e.g. SOD, CAT, and GPx) and non-enzymatic systems (e.g. vitamins C, E and GSH). Due to its location in mitochondria and its position in the antioxidant chain, SOD is usually considered as particularly important since even modest decreases in SOD are sufficient to provoke cell damage (Culotta 2000, Gort and Imlay 1998). Several trials have shown that improving glycemic control does not necessarily improve accompanying oxidative stress in diabetics (Seghrouchni 2002). Therefore, there is a need of a drug having hypoglycaemic as well as antioxidative activity. CVL (Fig.1) (2-methyl-5-(1-methylethyl)-phenol) was a predominant monoterpenic phenol which occurs in many essential oils of the family

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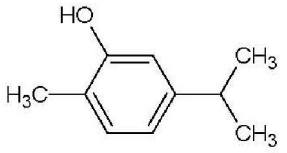


Fig.1. Structure of carvacrol

Labiatae including *Origanum*, *Satureja, Thymbra, Thymus*, and *Coridothymus* species (Kirimer 1995). Generally recognised as a safe food additive, CVL is used as a flavouring agent in sweets, beverages and chewing gum (Lagouri 1993). CVL has been reported to possess a wide variety of pharmacological properties including hepatoprotective (Aristatile *et al*., 2009), antiinflammatory (Hajhashemi *et al*., 2002), antioxidant (Yanishlieva *et al*., 1999), antitumour (Evangelou *et al*., 1997), antimicrobial activity (Sokmen *et al*., 2004). RSG (Fig. 2) was a synthetic peroxisome proliferator-activated receptor γ (PPAR-γ) agonist belonging to the thiazolidinedione class of compounds and is prescribed as an antidiabetic drug.

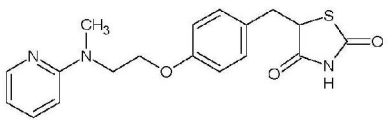


Fig. 2. Structure of rosiglitazone

It improves insulin sensitivity and lowers blood glucose and lipid levels. Yu *et al* reported that RSG potently suppressed the production of ROS and lipid peroxidation. Also, they found that treatment with RSG protect the central nervous system from oxidative damage in epileptic rats through enhanced antioxidative activity of SOD and GSH, together with decreased expression of heme oxygenase-1 in the hippocampus (Yu *et al*., 2008). The current study was designed to

investigate the combined effect of CVL and RSG on oxidative stress and antioxidant defense system in HFD induced C57BL/6J mice.

MATERIALS AND METHODS

Animals and diet

Male C57BL/6J (5 week old) mice with a body weight ranging from 20 to 30 g, were procured from National Institute of Nutrition, Hyderabad. The mice were maintained in an air conditioned room (25±1 °C) with a 12 h light/12 h dark cycle in the Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, food and water provided *ad libitum* to all the animals. The study protocol was approved by the Institutional Animals Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg. No. 160/1999/CPCSEA, Proposal number: 819), Annamalai University, Annamalainagar. Animals were fed initially with standard diets for 2 weeks. The compositions of experimental diets were shown in Table 1. Then these mice were assigned to one of six groups with 10 mice in each group: CVL and RSG were administered as suspension directly into the stomach using a intragastric tube in the morning for last 5 weeks morning at 9 am the former, by mixing with vehicle 0.5% dimethyl sulfoxide (DMSO), and the latter, in drinking water.

Table 1. Composition of normal and high fat diet

Ingredients	Standard pellet diet	High fat diet
Protein	21.1%	21.1%
Fat	5.1%	5.1% + 34.9% beef tallow
Carbohydrate	60.0%	60.0%
Fiber	3.9%	3.9%
Minerals	7.9%	7.9%
Vitamins	2.0%.	2.0%

Chemicals

CVL was purchased from Sigma Aldrich (St. Louis, Missouri, USA). RSG (Windia) was purchased from Glaxo SmithKline, Mumbai, India. All other chemicals used in this study were of analytical grade, obtained from E. Merck or HIMEDIA, Mumbai, India.

Experimental design

The mice were fed HFD for 10 weeks and then the animals were randomly divided into six groups of ten mice each as given below. CVL dissolved in 0.5% DMSO and RSG dissolved in water were administered through a gavage once in a day in the morning for 35 days.

Group I: Control (0.5% DMSO)
Group II: Control+ CVL (20 mg/kg BW) + RSG (4 mg/kg BW)
Group III: HFD
Group IV: HFD + CVL (20 mg/kg BW)
Group V: HFD + RSG (4 mg/kg BW)
Group VI: HFD + CVL (20 mg/kg BW) + RSG (4 mg/kg BW)

Biochemical estimations

The levels of TBARS, LOOH and CD were estimated by the methods of Niehaus and Samuelson (1998), Jiang *et al*. (1992), Rao and Recknagel (1968), respectively. The levels of vitamin C, vitamin E and GSH were estimated by the methods of Roe and Kuether (1943), Baker *et al*. (1980), Ellman *et al*. (1959), respectively. The protein content was determined by the method of Lowry *et al*. (1951). SOD, CAT, GPx and GST were assayed with the methods of Kakkar *et al*. (1984), Sinha (1972), Rotruck *et al*. (1973) and Habig *et al*. (1974) respectively.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using Statistical Package for the Social Science (SPSS, Chicago, IL) software version 11.5. Values were given as means ± S.D. for six samples pooled from the blood of ten mice in each group and for tissues from six mice. The limit of statistical significance was set at $p \leq 0.05$.

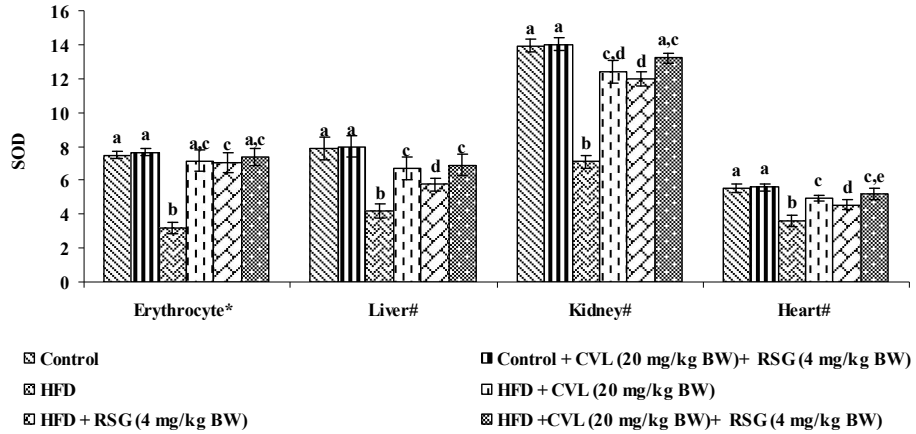
RESULT

The levels of TBARS, LOOH and CD in the plasma and tissues of diabetic and control mice were presented in the Table 2. HFD-fed mice had elevated levels of TBARS, LOOH and CD in the plasma and tissues, combination of CVL and RSG significantly decreased the concentrations of TBARS, LOOH and CD in the plasma and tissues (liver, kidney and heart) when compared to individual treatment with CVL or RSG. Figures 3, 4, 5 and 6 show the activities of SOD, CAT, Gpx and GST in the erythrocyte and tissues of diabetic and control mice. HFD-fed mice had decreased activities of SOD, CAT, Gpx and GST in the erythrocyte and tissues as compared with normal control mice. CVL or RSG lone treatment, significantly increased the activities of these enzymatic antioxidants (SOD, CAT, Gpx and GST) and combination of CVL and RSG further significantly influenced towards normality. The levels of vitamin C, E and GSH in the plasma and tissues of diabetic and control mice were given in Figures 7, 8 and 9. HFD-fed mice showed decreased levels of vitamin C, vitamin E and GSH in the plasma and tissues when compared with control mice.

Table 2. Effect of CVL and RSG on lipid peroxidation markers in the plasma and tissues of HFD-fed C57BL/6Jdiabetic mice

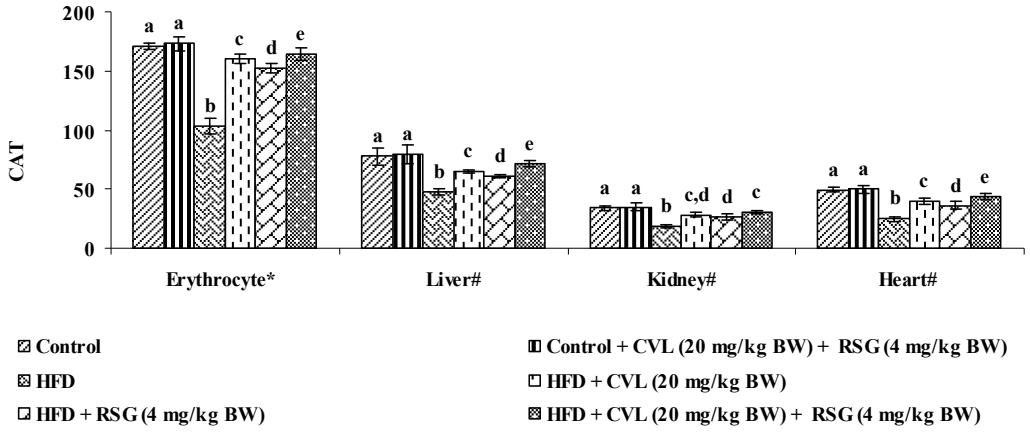
Groups		Control	Control + CVL (20 mg/kg BW) + RSG (4 mg/kg BW)	HFD	HFD + CVL (20 mg/kg BW)	HFD + RSG (4 mg/kg BW)	HFD + CVL (20 mg/kg BW) + RSG (4 mg/kg BW)
TBARS	Plasma (mmol/dL)	0.15 ± 0.01^{a}	0.14 ± 0.01^{a}	0.39 ± 0.02^{b}	0.27 ± 0.01^{c}	0.27 ± 0.01^{c}	0.19 ± 0.01^{d}
	Liver (mmol/100 g wet tissue)	0.85 ± 0.05^{a}	0.83 ± 0.04^{a}	3.80 ± 0.26^{b}	1.68 ± 0.07^{c}	1.54 ± 0.13^{c}	1.10 ± 0.10^{d}
	Kidney (mmol/100 g wet tissue)	1.51 ± 0.15^{a}	1.47 ± 0.15^{a}	3.87 ± 0.22^{b}	2.24 ± 0.20^{c}	2.13 ± 0.15^{c}	1.76 ± 0.17^{d}
	Heart (mmol/100 g wet tissue)	0.50 ± 0.03^{a}	0.46 ± 0.03^{a}	3.13 ± 0.21^{b}	0.93 ± 0.07^{c}	0.81 ± 0.06^{d}	0.65 ± 0.04^{e}
LOOH	Plasma (mmol/dL)	8.36 ± 0.51^{a}	8.18 ± 0.43^{a}	30.94 ± 1.74^{b}	13.74 ± 0.63^{c}	13.21 ± 0.50^{c}	10.83 ± 0.58^{d}
	Liver (mmol/100 g wet tissue)	80.58 ± 3.32^{a}	79.21 ± 2.67^{a}	138.67 ± 6.15^{b}	109.51 ± 9.49^{c}	107.13 ± 9.58^{c}	91.65 ± 5.37^{d}
	Kidney(mmol/100 g wet tissue)	72.91 ± 2.86^{a}	71.36 ± 3.11^{a}	144.03 ± 11.22^{b}	98.79 ± 7.02^{c}	94.63 ± 6.68^{c}	86.89 ± 4.32^{d}
	Heart (mmol/100 g wet tissue)	69.63 ± 3.74^{a}	68.44 ± 2.68^{a}	142.24 ± 5.25^{b}	83.92 ± 4.92^{c}	$82.13 \pm 4.52^{c,d}$	77.96 ± 4.17^{d}
CD	Plasma (mmol/dL)	0.61 ± 0.04^{a}	0.58 ± 0.05^{a}	1.54 ± 0.15^{b}	0.89 ± 0.04^{c}	0.84 ± 0.05^{c}	0.73 ± 0.05^{d}
	Liver(mmol/100 g wet tissue)	70.50 ± 2.12^{a}	69.5 ± 2.05^{a}	107.25 ± 6.46^{b}	87.75 ± 3.25^{c}	84.50 ± 4.09^{c}	74.25 ± 3.11^{d}
	Kidney (mmol/100 g wet tissue)	21.25 ± 1.75^{a}	20.25 ± 1.57^{a}	39.50 ± 2.81^{b}	25.25 ± 2.21^{c}	24.50 ± 2.05^{c}	22.01 ± 1.55^{a}
	Heart (mmol/100 g wet tissue)	38.00 ± 1.55^{a}	36.75 ± 2.27^{a}	78.75 ± 2.80^{b}	48.75 ± 2.07^{c}	$47.50 \pm 1.81^{c,d}$	45.25 ± 1.75^{d}

Values are means ± SD for 10 mice
Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)



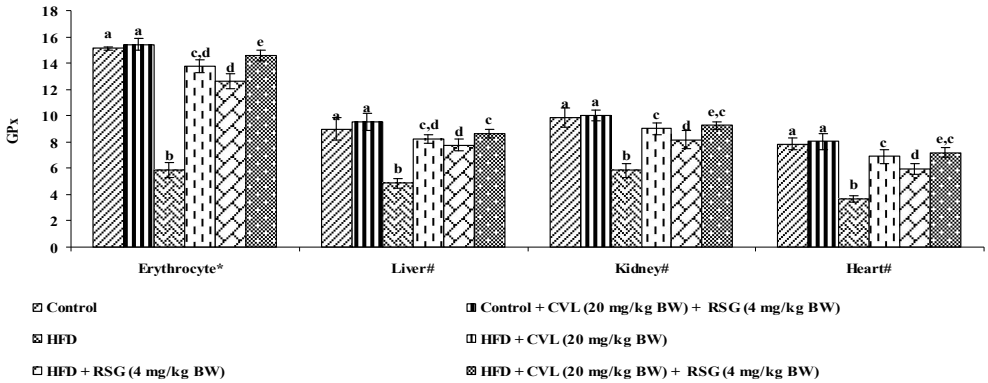
Values are means ± SD for six samples from 10 mice in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)
U = Enzyme concentration required for 50% inhibition of NBT reduction/minute.
U*/mg Hb*= In the erythrocyte
U#/mg protein= In the tissues

Fig. 3. Effect of CVL and RSG on the activity of superoxide dismutase in the erythrocyte and tissues of HFD-fed C57BL/6J diabetic mice



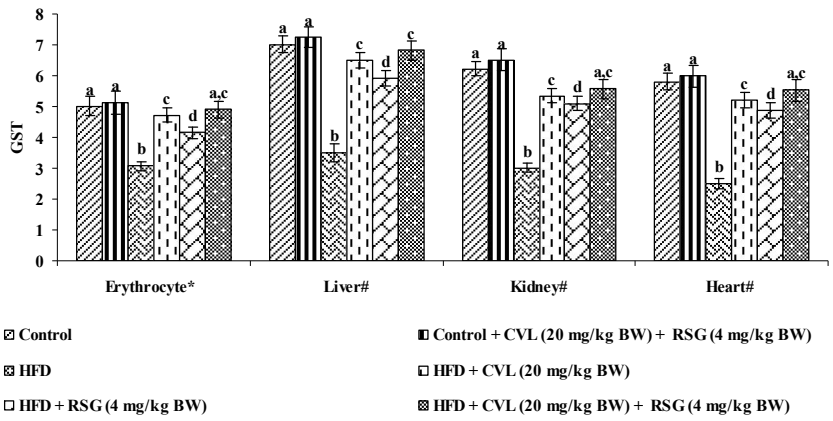
Values are means ± SD for six samples from 10 mice in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)
U- µmol of H_2O_2 consumed per minute.
U*/mg Hb*= In the erythrocyte
U#/mg protein= In the tissues

Fig.4. Effect of CVL and RSG on the activity of catalase in the erythrocyte and tissues of HFD-fed C57BL/6J diabetic mice



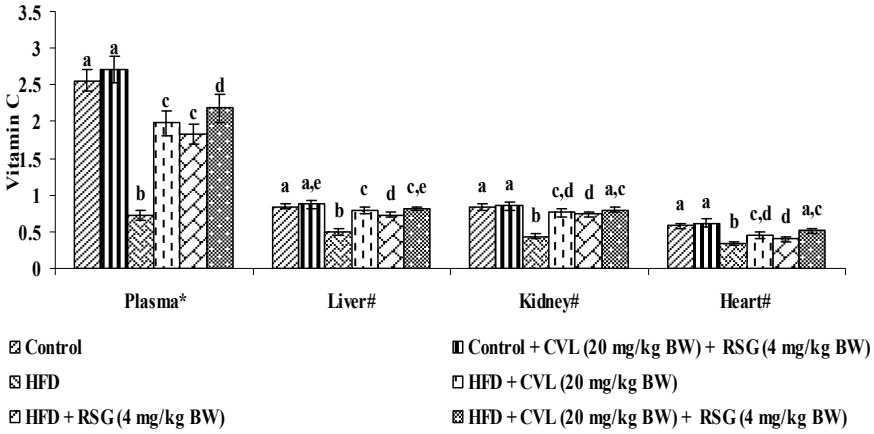
Values are means ± SD for six samples from 10 mice in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)
U- µg of GSH utilized per minute.
U*/mg Hb*= In the erythrocyte
U#/mg protein= In the tissues

Fig. 5. Effect of CVL and RSG on the activity of glutathione peroxidase in the erythrocyte and tissues of HFD-fed C57BL/6J diabetic mice



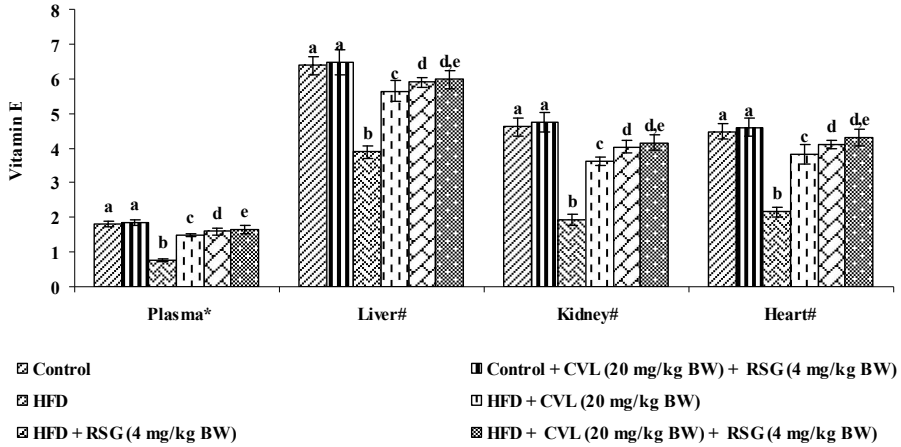
Values are means ± SD for six samples from 10 mice in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)
U = µg of CDNB conjugate formed/minute
U*/mg Hb*= In the erythrocyte
U#/mg protein= In the tissues

Fig. 6. Effect of CVL and RSG on the activity of glutathione-S- transferase in the erythrocyte and tissues of HFD-fed C57BL/6J diabetic mice



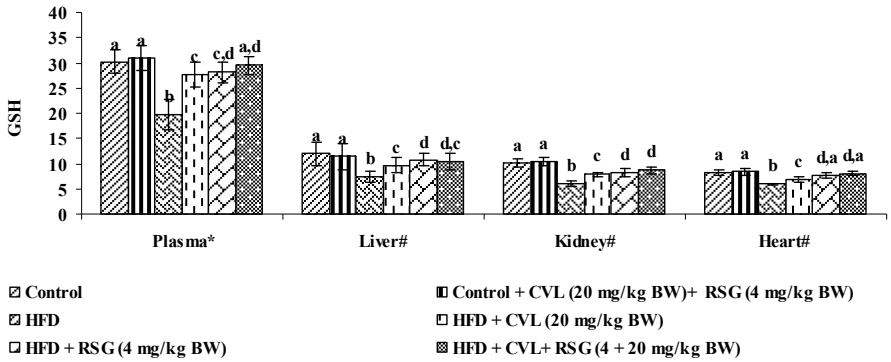
Values are means ± SD for six samples from 10 mice in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)
mg/dL*= In the plasma
µg/mg protein#= In the tissues

Fig. 7. Effect of CVL and RSG on vitamin C in the plasma and tissues of HFD-fed C57BL/6J diabetic mice



Values are means ± SD for six samples from 10 mice in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)
mg/dL*= In the plasma
µg/mg protein#= In the tissues

Fig. 8. Effect CVL and RSG on vitamin E in the plasma and tissues of HFD-fed C57BL/6J diabetic mice



Values are means ± SD for six samples from 10 mice in each group.
Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT)
mg/dL.*= In the plasma
µg/mg protein#= In the tissues

Fig.9. Effect of CVL and RSG on reduced glutathione in the plasma and tissues of HFD-fed C57BL/6J diabetic mice

Administration of CVL or RSG to HFD mice significantly increased the levels of vitamin C, vitamin E and GSH, respectively. Further more the combination of CVL and RSG restored the levels of vitamin C, vitamin E and GSH to near normality. Histopathological evaluation of liver in control group showed normal morphology of central vein (Fig. 10A). Morphological changes including hepatocytes with micro and macrovesicular fatty degeneration with dilated sinusoids were observed in the HFD-fed mice (Fig. 10 C). CVL and RSG treated mice recovered the above mentioned changes to near normal (Fig. 10 D, E & F).

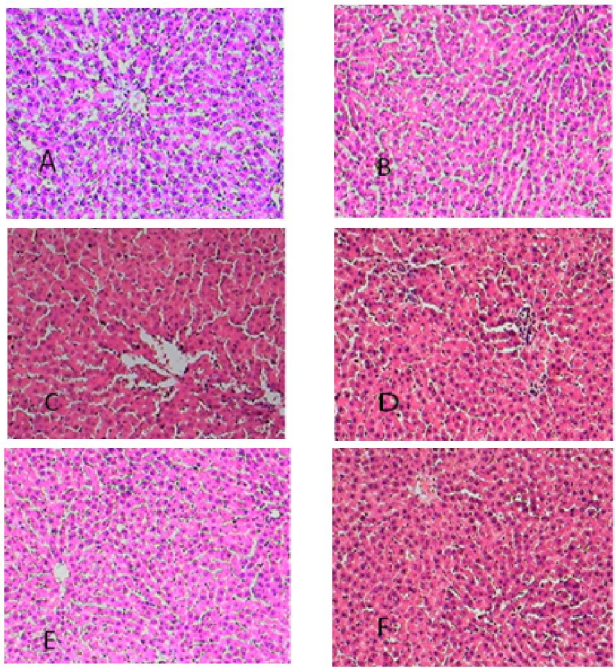


Fig. 10 Histopathological changes in liver

A) Control: Normal hepatocyte cellular architecture with normal central portal vein. B) Control + CVL + RSG: Central vein surrounded by normal hepatocytes. C) HFD: Moderate micro and macrovesicular fatty degeneration of liver with dilated sinusoids. D) HFD + CVL: Dilated vessels and sinusoids and inflammatory fatty infiltration. E) HFD + RSG: Central vein surrounded by normal hepatocytes cell fatty infiltration and sinusoidal dilatation. F) HFD + CVL + RSG: combined effect showing micro and macro vasicular steatosis central vein and reduced fatty infiltration.

Figure 11 illustrates the histopathological changes of kidney tissues of control and HFD fed diabetic mice. Control mice showed normal glomeruli and tubules (Fig. 11 A). Diabetic mice kidney revealed the vacuolar degeneration of renal tubular cells with displaced pycnotic nuclei (Fig. 11 C).

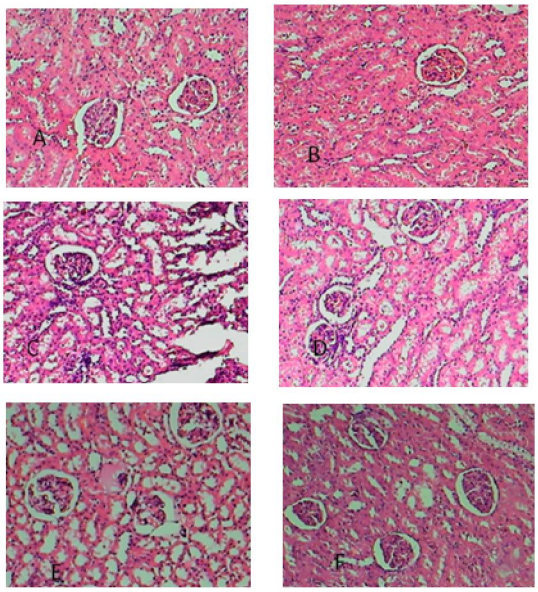


Fig. 11 Histopathological changes in kidney

A) Control: Normal renal tubular architecture and glomeruli. B) Control + CVL + RSG: combined effect showing normal tubular cells. C) HFD: Renal parenchyma showing vacuolar degeneration of renal tubular cells with displaced pycnotic nuclei. D) HFD + CVL: showing normal renal glomeruli and mild fatty infiltration. E) HFD + RSG: Renal glomeruli showing normal histology and mild fatty infiltration. F) HFD + CVL + RSG: combined effect showing normal renal glomeruli.

Administration of CVL and RSG to HFD-fed mice remarkably reduced the above changes and showing mild tubular dilation and normal glomeruli (Fig.11D, E & F).

DISCUSSION

A physiological balance between the generation and detoxification of free radicals was maintained by the normal activity of the antioxidant system. Oxidative stress in obesity may be an important pathogenic mechanism in obesity related metabolic syndrome (Furukawa *et al*., 2004). Possible mechanisms contributing to the obesity associated oxidative stress include increased oxygen consumption and subsequent radical production via mitochondrial respiration, diminished antioxidant capacity, increased fat deposition and cell injury leading to increased formation rates of radicals, such as superoxide anion and hydroxyl radical (Vincent *et al*., 2001). In particular, erythrocytes present in the blood are subjected to a continuous flux of superoxide anions and hydrogen peroxide, resulting from hemoglobin oxidation, and undergo oxidative stress induced by both external and internal environmental agents (Saggu and Kumar 2008). Oxidative stress can be defined as a state of imbalance toward

the factors that generate reactive oxygen radicals (e.g., superoxide or hydroxyl radicals) and away from the factors that protect cellular macromolecules from these reactants including antioxidants like SOD, CAT and GPx. The factors that generate (ROS) exist as products of normal cellular physiology as well as from various exogenous sources. Mitochondria were thought to be the source of most cellular ROS, specifically superoxide radicals. The reactions that generate ATP in the mitochondria require electrons from reduced substrates to be passed along the complexes of the electron transport chain. In the presence of molecular oxygen, electrons that leak from this process react and form the free radical superoxide. Superoxide anions were significant mediators in numerous oxidative chain reactions and were also a precursor to many other ROS (Halliwell and Gutteridge 1989). Other significant intracellular sources of ROS include NADPH oxidases (which generate superoxide), nitric oxide synthases (nitric oxide) and lipoxygenases (fatty acid hydroperoxides) (Halliwell and Gutteridge 1989). In addition, certain cell types within tissue systems may promote localized environments with elevated oxidative stress. For example, macrophages can produce localized oxidative stress as part of the inflammatory response (Federico *et al*., 2007). Thus, low levels of ROS were typical within both the cell and the higher order tissue and organ systems and some ROS (in particular superoxide and hydrogen peroxide) were required to support natural cellular function and regulate intracellular signaling (Perez-Matute *et al*., 2009). However, excess ROS production (or reduced ROS regulation) can severely impair the cell and lead to macromolecular damage, dysfunction and death.

Phenolic compounds were commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kahkonen *et al*. 1999). The ability of CVL to enhance the levels of antioxidants along with its antilipid peroxidative activity suggests that this compound might be potentially useful in counteracting free-radical-mediated tissue damage caused by hepatotoxicity. CVL is a major component of the essential oil of Oregano (Rodrigues *et al*., 2004). Generally recognized as a safe food additive, the main active ingredients of the essential oil were thymol and CVL, with antioxidative, antimicrobial and antifungal effects (Aeschbach *et al*., 1994, Proestos *et al*., 2005). RSG protect the central nervous system from oxidative damage in epileptic rats through enhanced antioxidative activity (Yu *et al*. 2008). Oxidative stress produces deleterious effects by initiating lipid peroxidation directly or by acting as second messengers for the primary free radicals that initiate lipid peroxidation (Das 2002). The TBARS value was measured as biomarkers of lipid peroxidation. Lipid peroxidation and protein oxidation in microsome were significantly higher in the HFD group compared with control group. The marked increase in the production of TBARS could be due to the superoxide radical overload, indicating the presence of oxidative stress and a subsequent increase in the production of hydrogen peroxide. This was consistent with previous reports that high-fat diet induces critical oxidative damage in the liver (Schrauwen 2007, Prasamthi *et al*., 2005). In our laboratory, Aristatitle *et al.* (2010) reported that CVL posses antioxidant effect. In this study, treatment with CVL and RSG reduced the lipid peroxidation level significantly, whereas CVL and RSG combination was found to be more effective to over come oxidative stress when compare to individual treatment with CVL and RSG. The anti-oxidative markers, SOD, GPx, and CAT have been established and utilized in the evaluation of oxidative stress in animal model with hypertension, hyperlipidemia, obesity, and diabetes (Saiki *et al*., 2007). SOD is an important defense enzyme which catalyses the dismutation of superoxide radicals (McCord *et al*., 1971). CAT is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from hydroxyl radicals (Chance *et al*., 1952). Therefore reduction in the activity of these enzymes (SOD, CAT) may result in a number of deleterious effects due to the accumulation of superoxide anion and hydrogen peroxide (Searle and Willson 1980). GPx plays a pivotal role in H_2O_2 catabolism and in the detoxification of endogenous metabolic peroxides and hydroperoxides which catalyses GSH (Eaton, 1991). GST is a family of isoenzymes which participate in the conjugation of toxic electrophiles with GSH (Jakoby 1988). The present study also showed a significant increase in lipid peroxidation and decrease in the antioxidant enzyme activity of SOD, CAT, GPx and GST in agreement with Coskun *et al*., 2005. In our study, combined treatment with CVL and RSG showed increased level of SOD, CAT, GPx and GST significantly and brought the above enzymatic antioxidant to near normality. Apart from the enzymatic antioxidants, non-enzymatic antioxidants such as vitamin C, vitamin E, reduced glutathione play an excellent role in preventing the cells from oxidative threats. Vitamin E is the most ancient antioxidant in the lipid phase (Ingold *et al*., 1987). In our study, vitamin C and E were decreased in diabetic mice, which could be due to increased membrane damage by reactive oxygen species. Treatment with CVL and RSG brought vitamin E, vitamin to near normal levels which could be as a result of decreased membrane damage as evidenced by decreased lipid peroxidation. Glutathione (GSH) is essential for the cellular antioxidant defense response and acts as an essential cofactor for antioxidant enzymes (Kidd, 1997). It was also observed in an untreated diabetic condition, free hydroxyl radicals increase, the activities of antioxidant enzymes decreased, resulting in the reduction in GSH level. This was accompanied by an increase in the levels of lipid peroxidation markers resulting in oxidative stress (Li *et al*., 2007). In, our study CVL and RSG combined treatment brought the GSH activity to near normal in our study, when compare to individual treatment the combination CVL and RSG was found to be more effective by increasing GSH activity to near normalcy. HFD-fed mice liver showed moderate micro and macrovesicular fatty degeneration with dilated sinusoids. HFD-fed mice kidney showed vacuolar degeneration of renal tubular cells with displaced pycnotic nuclei. Administration of CVL and RSG brought the above-mentioned changes in liver and kidney to near normal architecture.

Conclusion

Thus, combined effect of CVL and RSG shows good antioxidant property, as evidenced by increased antioxidants status and decreased lipid peroxidation, which may protect from the risk of diabetic complications.

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REFERENCES

Aeschbach R., Löliger J., Scott B. C., Murcia A., Butler J., Halliwell B., Aruoma O. I. (1994): Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem Toxicol 32, 31-36

Aristatile B., Al-Numair K. S., Al-Assaf A. H., Veeramani C., Pugalendi K. V. (2010): Protective Effect of Carvacrol on Oxidative Stress and Cellular DNA Damage Induced by UVB Irradiation in Human Peripheral Lymphocytes. J Biochem Molecular Toxicol. 32, 1-11

Aristatile B., Al-Numair K. S., Veeramani C., Pugalendi K. V. (2009): Effect of carvacrol on hepatic marker enzymes and antioxidant status in D-galactosamine-induced hepatotoxicity in rats. Fundam Clin Pharmacol 23, 757-765

Baker H. (1980): Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr Rep Int 21,531-536

Chance B., Greenstein D. S., Roughton F. J. (1952): The mechanism of catalase action. I. Steady-state analysis. Arch Biochem Biophys 37, 301-321

Coskun O., Kanter M., Korkmaz A., Oter S. (2005): Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol Res 51, 117-123

Culotta V. C. (2000): Superoxide dismutase, oxidative stress, and cell metabolism. Curr Top Cell Regul 36, 117-132

Das U. (2002): A radical approach to cancer. MedSciMonit8,RA79-92

Eaton J. W. (1991): Catalases and peroxidases and glutathione and hydrogen peroxide: mysteries of the bestiary. J Lab Clin Med 118, 3-4

Ellman G. L. (1959): Tissue sulphydryl groups. Arch Biochem Biophys 82, 70-77

Evangelou A., Kalpouzos G., Karkabounas S., Liasko R., Nonni A., Stefanou D., Kallistratos G. (1997): Dose-related preventive and therapeutic effects of antioxidants-anticarcinogens on experimentally induced malignant tumors in Wistar rats. Cancer Lett 115, 105-111

Fang Y. Z., Yang S., Wu G. (2002): Free radicals, antioxidants, and nutrition. Nutrition 18, 872-879.

Federico A., Morgillo F., Tuccillo C., Ciardiello F., Loguercio C. (2007): Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer 121, 2381-2386

Furukawa S., Fujita T., Shimabukuro M., Iwaki M., Yamada Y., Nakajima Y., Nakayama O., Makishima M., Matsuda M., Shimomura I. (2004): Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114, 1752-1761

Gort A.S., Imlay J.A. (1998): Balance between endogenous superoxide stress and antioxidant defenses. J Bacteriol 180, 1402-1410

Habig W. H., Pabst M. J., Jakoby W. B. (1974): Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249, 7130-139

Hajhashemi V., Ghannadi A., Pezeshkian S. K. (2002): Antinociceptive and anti-inflammatory effects of *Satureja hortensis* L. extracts and essential oil. J Ethnopharmacol 82,83-87

Halliwell B., Gutteridge J. M. C. (1989): Free radicals in biology and medicine. Oxford University Press, Oxford, UK

Hayden M. R, Tyagi S. C. (2002): Islet redox stress: the manifold toxicities of insulin resistance, metabolic syndrome and amylin derived islet amyloid in type 2 diabetes mellitus. JOP J Pancreas (Online) 3, 86-108

Ingold K. U., Webb A. C., Witter D., Burton G. W., Metcalfe T. A., Muller D. P. (1987): Vitamin E remains the major lipid-soluble, chain-breaking antioxidant in human plasma even in individuals suffering severe vitamin E deficiency. Arch Biochem Biophys 259, 224-225

Jakoby W. B. (1988): Detoxification, conjugation and hydrolysis in liver biology and pathology In: (Eds Aris I. M. and Jakoby W. B). Raven Press Inc, pp. 375-385, New York

Jiang Z. Y., Hunt J. V., Wolff S.P. (1992): Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 202, 384-389

Kähkönen M. P., Hopia A. I., Vuorela H. J., Rauha J. P., Pihlaja K., Kujala T. S., Heinonen M. (1999): Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 47, 3954-3962

Kakkar P., Das B., Viswanathan P. N. (1984): A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 21, 130-132

Kidd P. M. (1997): Glutathione: Systemic protectant against oxidative and free radical damage. Alt Med Review 2, 155-176

Kirimer N, Baser K. H. C, Tumen G. (1995): Carvacrol rich plants in Turkey. Chem Nat Compd 31, 37–42.

Lagouri V., Bleskas G., Tsimidou M, Kokkini S., Boskou D. (1993): Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece.Z Lebens Unter Fors 197, 20–23.

Li F., Ji L., Luo Y., Oh K. (2007): Hydroxyl radical generation and oxidative stress in Carassius auratus liver as affected by 2,4,6-trichlorophenol. Chemosphere 67, 13-19

McCord J. M., Keele B. B., Fridovich I. (1971): An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc Natl Acad Sci U S A 68, 1024-1027

Niehaus W. G., Samuelsson B. (1968): Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 6, 126-130

Pérez-Matute P., Zulet M. A., Martínez J. A. (2009): Reactive species and diabetes: counteracting oxidative stress to improve health. Curr Opin Pharmacol 9, 771-779

Prasamthi K., Muralidhara, Rajini P. S. (2005): Fenvalerate-induced oxidative damage in rat tissues and its attenuation by dietary sesame oil. Food Chem Toxicol 43, 299-306.

Proestos C., Chorianopoulos N., Nychas G. J., Komaitis M. (2005): RP-HPLC analysis of the phenolic compounds of plant extracts. investigation of their antioxidant capacity and antimicrobial activity. J Agric Food Chem 53, 1190-1195

Rao K. S., Recknagel R. O. (1968): Early onset of lipoperoxidation in rat liver after carbon tetrachloride administration. Exp Mol Pathol 9, 271-278

Rodrigues F. A., McNally D. J., Datnoff L. E, Jones J. B., Labbé C., Benhamou N., Menzies J. G., Bélanger R. R. (2004): Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. Phytopathology 94, 177-183

Roe J. H., Kuether C. A., (1943): Detection of ascorbic acid in whole blood and urine through 2, 4-DNPH derivative of dehydroascorbic acid. J Biol Chem 47, 399-407

Rotruck J. T., Pope A. L., Ganther H. E., Swanson A. B, Hafeman D. G., Hoekstra W.G. (1973) : Selenium: biochemical role as a component of glutathione peroxidase. Science 179, 588-590.

Saggu S., Kumar R. (2008): Effect of seabuckthorn leaf extracts on circulating energy fuels, lipid peroxidation and antioxidant parameters in rats during exposure to cold, hypoxia and restraint (C-H-R) stress and post stress recovery. Phytomedicine 15,437-446

Saiki R., Okazaki M., Iwai S., Kumai T., Kobayashi S., Oguchi K. (2007): Effects of pioglitazone on increases in visceral fat accumulation and oxidative stress in spontaneously hypertensive hyperlipidemic rats fed a high-fat diet and sucrose solution. J Pharmacol Sci 105, 157-167

Schrauwen P. (2007): High-fat diet, muscular lipotoxicity and insulin resistance. Proc Nutr Soc 66, 33-41.

Searle A. J., Willson R. L. (1980): Glutathione peroxidase: effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. Int J Radiat Biol Relat Stud Phys Chem Med 37, 213-217

Seghrouchni I., Drai J., Bannier E., Rivière J., Calmard P., Garcia I., Orgiazzi J., Revol A. (2002): Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. Clin Chim Acta 321, 89-96

Sinha K. A. (1972): Colorimetric assay of catalase. Anal Biochem 47, 389-394.

Sökmen M., Serkedjieva J., Daferera D., Gulluce M., Polissiou M., Tepe B., Akpulat H. A., Sahin F., Sokmen A. (2004): *Invitro* antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*. J Agric Food Chem 52, 3309-3312

Vincent H. K., Powers S.K., Dirks A. J., Scarpace P. J. (2001): Mechanism for obesity-induced increase in myocardial lipid peroxidation. Int J Obes Relat Metab Disord 25, 378-388

WHO Obesity and Overweight (Fact sheet 311), World Health Organization (http://www.who.int/mediacentre/factsheets/fs311 /en/ index.html), 2006.

Yanishlieva N. V., Marinova E. M., Gordon M. H., Reneva VG (1999): Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem64,59–66

Yu X., Shao X. G, Sun H., Li Y. N., Yang J., Deng Y. C., Huang Y. G. (2008): Activation of cerebral peroxisome proliferator-activated receptors gamma exerts neuroprotection by inhibiting oxidative stress following pilocarpine-induced status epilepticus, Brain Res 20, 146-158

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