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The Effects of Dietary Supplementation of Different Amount of *Yucca schidigera* Powder (Sarsaponin 30®) on Blood and Tissue Antioxidant Defense Systems and Lipid Peroxidation in Rats

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Abstract: The study was aimed to determine the possible protective role of diet suplementation of *Yucca schidigera* (Ys) powder against basal oxidative damage in blood and some tissue on rats. The rats were divided into 3 groups: Control, Ys1 and Ys2. Control group was fed by Standart Rat Feed (SRF). The other groups, Ys1 and Ys2 were fed *ad libitum* by SRF +100 ppm Ys powder (Sarsaponin 30®), SRF + 200 ppm Ys powder (Sarsaponin 30®), respectively for 4 weeks during the study. MDA levels in blood and kidney of the rats significantly decreased in Ys1 and Ys2 groups compared to control. Whereas, liver MDA levels of Ys1 and Ys2 groups didn't show any significant change. The kidney GSH concentrations were significantly increased in the Ys2 group compared to control. Blood and liver GSH concentrations between groups did not differ. Consequently, 100 and 200 ppm supplementation of Ys powder to the diets of rats decrease the blood and kidney MDA levels and increase the kidney GSH concentrations. It is thought that Ys can be used effectively as an antioxidant suplement. Moreover, the usage of the plant, did not affect the liver MDA and GSH as well as blood GSH levels.

Key words: Yucca schidigera, antioxidant, free radicals, tissue

INTRODUCTION

Oxygen is a dangerous friend. Overwhelming evidence indicates that oxidative stress can lead to cell and tissue injury. However, the same free radicals that are generated during oxidative stress are produced during normal metabolism and thus are involved in both health and disease (Packer *et al*., 2004). Under normal circumstances, the Reactive Oxygen species (ROS) generated are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and the antioxidants present. However, owing to ROS overproduction or inadequate antioxidant defense, this equilibrium is hampered favouring the ROS upsurge that culminates in oxidative stress. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Farber, 1994; Kaur *et al*., 2006). This oxidative damage is a crucial etiological factor implicated in several chronic diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in the ageing process (Hogg, 1998; Pong, 2003; Kaur *et al*., 2006).

During the last decade, numerous *in vitro* and *in vivo* studies have suggested that plants have numerous potentially beneficial medicinal properties. So, that the use of herbal medications has soared over the last decade. In 1997, an estimated 12% of the population used over-the-counter herbal products, resulting in billions of dollars in sales for the herbal industry (Packer *et al*., 2004). It is well known that many natural substances in plants have antioxidant activity. The antioxidants may mediate their effect by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions (Robak and Marcinkiewicz, 1995). Recently, some synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have been suspected to be dangerous to health (Safer and Al-Naghamigh, 1999). Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant ability which could be used in medicine and additives to nutriceuticals (Thomes and Wade, 2001).

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Yucca schidigera (Ys) is an herbaceous plant of the lily family, native to the deserts of the south-western United States and northern Mexico. According to folk medicine, yucca extracts have anti-arthritic and anti-inflammatory effects. The plant contains several physiologically active phytochemicals. It is a rich source of steroidal saponins and is used commercially as a saponin source. Saponins have diverse biological effects, including anti-protozoal activity. It has been postulated that saponins may have anti-arthritic properties by suppressing intestinal protozoa which may have a role in joint inflammation (Cheeke *et al*., 2006; Fidan and Dundar, 2007).

The present study has therefore been undertaken to determine the possible protective role of diet suplementation of Ys powder against basal oxidative damage on rats.

MATERIALS AND METHODS

Ys powder (Sarsaponin 30®), incorporating in the trial rations of our study, were provided from the firm Desert King International (San Diego, CA, USA). According to the information obtained from the firm, the preparations were produced without losing the phytochemicals they contained as 100% plant powder whereas moreover, they contained also Sarsaponin 30®>8% steroidal saponin. Chemicals used in the study were purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co. St. Louis, MO, USA).

Male Sprague-Dawley rats, weighing about 180-250 g were used. They were housed under standard conditions of temperature (23±2°C), humidity and dark-light cycle (lights on from 6:00 am-6:00 pm). The animals were maintained on standard rat feed supplied by Bil- Yem Ltd. (Turkey). Tap water was available ad libitum. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the University of Afyon Kocatepe Animal Ethics Committee (050606). The rats were randomly divided into 3 experimental groups: Control (C) (n = 15); experimental group I (Ys1) (n = 15) and experimental group II (Ys2) (n = 15). C group was fed by standart rat feed (SRF). The other experimental groups, Ys1 and Ys2 were fed *ad libitum* by SRF +100 ppm Ys powder, SRF +200 ppm Ys powder respectively for 4 weeks during the study.

At the end of the experimental period, the rats were anaesthetized and killed by cervical dislocation. Blood samples were taken into heparinized tubes in the fasting state in all subjects from heart. Two milliliters of blood were immediately pipetted into another tube to measure malondialdehyde (MDA) and reduced glutathione (GSH). The tissues (liver and kidney) were removed immediately and washed in ice-cold saline. Tissues were homogenized 1:40 w/v in 0.1M phosphate buffer, pH 7.4, containing 1 mM EDTA. After centrifugation at 18 000 × g for 15 min at 4°C, the supernatant was extracted and kept at -30°C in advance of assays. In the tissue homogenates the levels of MDA and total GSH were assayed.

MDA levels, an index of lipid peroxidation, were measured by the double heating method of Draper and Hadley (1990). The method is based on spectrophotometric measurement of the purple color generated by the reaction of TBA with MDA. For this purpose, 2.5 mL of trichloroacetic acid solution (10%, w/v) was added to 0.5 mL whole blood in each centrifuge tube and tubes were placed in a boiling water bath for 15 min. After cooling to room temperature, the tubes were centrifuged at 1000 g for 10 min and 2 mL of each sample supernatant was transferred to a test tube containing 1 mL of TBA solution (0.67%, w/v). Each tube was then placed in a boiling water bath for 15 min. After cooling to room temperature, the absorbance was measured at 532 nm by using the Shimadzu UV 1601 spectrophotometer. The concentration of MDA was calculated based on the absorbance coefficient of the TBA-MDA complex ($\varepsilon = 1.56 \times 105/cm/M$) and it was expressed as nmol mg^{-1}.

The levels of MDA in tissues was determined according to the method described by Okhawa *et al.* (1979). In this method, MDA reacts with thiobarbituric acid to form a colored complex that has maximum absorbance at 532 nm. MDA levels as nmol g^{-1} wt was expressed.

The erythrocyte and tissues GSH concentration was measured using the method described by Beutler *et al.* (1963). Briefly, 0.2 mL fresh erythrocyte pellet or supernatant was added to 1.8 mL distilled water. Precipitating solution of 3 mL (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 mL distilled water) was mixed with haemolysate. The mixture was allowed to stand for approximately 5 min and then filtered (Whatman No. 42). Filtrate of 2 mL was taken and added into another tube and then 8 mL of the phosphate solution (0.3 M disodium hydrogen phosphate) and 1 mL DTNB were added. A blank was prepared with 8 mL of phosphate solution; 2 mL diluted precipitating solution (3 parts to 2 parts distilled water) and 1 mL DTNB reagent. A standard solution of the GSH was prepared (40 mg/100 mL). The optical density was measured at 412 nm in the spectrophotometer. Results were communicated as mg g^{-1} wt, for tissue or mg dL^{-1}, for blood.

All data were presented as mean±SE for parametric variables. Parametric variables were compared using one-way analysis of variance with post-hoc analysis using the Duncan test. Data were analyzed using the SPSS® for

Windows computing program (Version 10.0) and $p<0.05$, was considered statistically significant (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The results of all determination for the experimental and the control group tissues and blood are given in Table 1, Fig. 1 and 2.

Active oxygen species and oxygen radicals are common products of cellular metabolism. Antioxidant defense system prevents free radical formation or limits their damaging effects. These comprise enzymes to decompose peroxides, proteins to sequester transition metals and range of compounds to 'scavenge' free radicals. Free radicals are continuously formed in cells either as accidental by-products of metabolism or deliberately during, for example, phagocytosis. The most important reactant in free radical biochemistry in aerobic cells is oxygen and its radical derivatives (superoxide and hydroxyl radical), hydrogen peroxide and transition metals. The two main molecular components of the membrane are lipids and proteins. As lipids are prone to oxidation of unsaturated bonds, it is perhaps reasonable to advocate lipid peroxidation as a significant event in the development of membrane damage (Yagi, 1993). The impact which free radicals make on lipids is named as Lipid Peroxidation (LP). LP is a complicated radical chain reaction leading to the formation of various products including lipid hydroperoxides, conjugated dienes and malondialdehyde. Detection of lipid hydroperoxides and conjugated dienes and thiobarbituric acid-reactive substances (TBARS) such as MDA, are often applied to the study of lipid peroxidation reactions (Diplock, 1994; Enginar *et al*., 2006). Since, membrane phospholipids are major targets of oxidative damage, lipid peroxidation is often the first parameter analyzed for proving the involvement of free radical damage. Thus, the presence of MDA is considered as an indicator of free-radical damage through membrane lipid peroxidation (Katz *et al*., 1996; Enginar *et al*., 2006).

Under normal conditions, numerous cellular antioxidant systems exist to defend against oxidant stress and maintain the redox balance of the cell. ROS are cleared from the cell by enzymatic systems including superoxide dismutases, catalase and glutathione peroxidase, or the nonenzymatic system including alpha-tocopherol, ascorbic acid, glutathione and uric acid. Glutathione peroxidase plays an important role as defense mechanism in mammals, against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate. In addition, to its role as a substrate in GSH redox cycle, glutathione, also act as a direct endogenous scavenger of hydroxyl radicals, involved in detoxification and metabolism of a number of substances in the liver (Sen, 2000). As a consequence of GSH deficiency, a number of related functions may be impaired such as a decrease in reducing

Table 1: Effects of Ys powder on GSH and MDA content in blood and various tissues of rats

	Parameters	Control $\bar{x}$ ±SE	Ys1 $\bar{x}$ ±SE	Ys2 $\bar{x}$ ±SE
Blood	GSH (mg dL^{-1})	21.03±1.77	22.26±1.17	23.75±1.21
	MDA (nmol mL^{-1})	2.29±0.16^{a*}	1.77±0.10^{b*}	1.7±0.14^{b*}
Liver	GSH (mg g^{-1})	14.16±0.79	14.24±1.16	15.68±1.96
	MDA (nmol g^{-1})	6.7±0.79	4.83±0.74	5.36±0.39
Kidney	GSH (mg g^{-1})	6.66±0.98^{b*}	8.22±0.56^{b*}	11.43±1.25^{a*}
	MDA (nmol g^{-1})	14.08±0.29^{a*}	11.63±0.61^{b*}	12.43±0.4^{b*}

Values are shown as mean±S.E. Values with different letters show statistically significant differences (*:$p<0.05$)

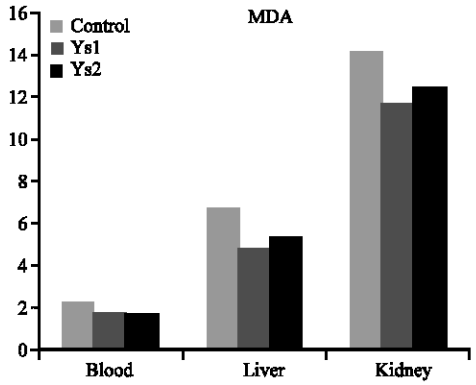

Fig. 1: The marker of lipid peroxidation MDA levels in the blood and kidney of the rats significantly decreased in Ys1 and Ys2 groups compared to control group ($p<0.05$). Whereas, liver MDA levels of Ys1 and Ys2 groups didn't show any significant change

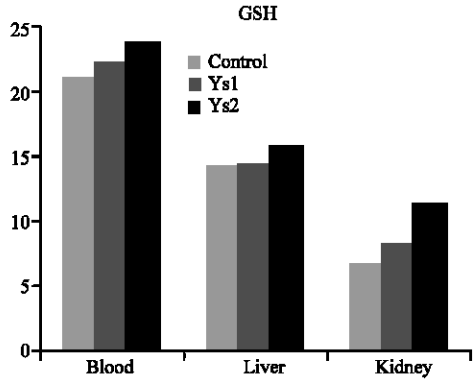

Fig. 2: The kidney GSH concentrations were significantly increased in the Ys2 group compared to control group ($p<0.05$). Blood and liver GSH concentrations between groups did not differ

capacity, protein biosynthesis, immune function, accumulations of lipid peroxidation products and detoxification capacity. A reduced detoxification capacity in the liver may lead to the accumulation of hepatotoxic metabolites leading to liver damage (Hayes and McLellan, 1999; Sen, 2000).

The use of plants and parts of part for therapeutic purposes has a long history. Previous studies demonstrated that Ys contains antioxidant principles (Olas *et al*., 2002; Piacente *et al*., 2004) and could limit the extent of lipid peroxidation (Aslan *et al*., 2005). Yucca is also a rich source of polyphenolics, including resveratrol and a number of other stilbenes (yuccaols A, B, C, D and E). These phenolics have anti-inflammatory activity. They are inhibitors of the nuclear transcription factor NFkappaB. NFkB stimulates synthesis of inducible Nitric Oxide Synthase (iNOS), which causes formation of the inflammatory agent nitric oxide. Yucca phenolics are also anti-oxidants and free-radical scavengers, which may aid in suppressing reactive oxygen species that stimulate inflammatory responses (Cheeke *et al*., 2006; Fidan and Dundar, 2007). The ability of resveratrol to inhibit LP has well been documented in interesting and recently published studies. For example, Leonard *et al.* (2003) demonstrated reaction the inhibitory effect of resveratrol against membrane LP in RAW 264.7 cells by exposure to OH radicals generated from the Fenton. Similarly, Sun *et al.* (1997) demonstrated a significant protective effect of resveratrol from oxidative stress in rat adrenal pheochromocytoma cells exposed to lipid oxidation induced by iron and ethanol. Resveratrol has also been shown to scavenge peroxyl and hydroxyl radicals in reperfused postischemic isolated rat hearts, reduce infarct size and to reduce the formation of MDA (Ray *et al*., 1999; Sato *et al*., 2000).

Sur *et al.* (2001) demonstrated the antioxidant properties of saponin containing plants. Moreover, they reported that although adding Ys powder to the ration didn't change the blood MDA levels, this increased the blood GSH levels and the total antioxidant capacity. Inspite of this Fidan and Dündar (2008) reported that blood MDA levels could be decreased by 100 ppm of Ys powder supplementation in diets of diabetic animals. Piacente *et al.* (2004) suggested that the significant activities exhibited by the phenol fraction and its constituents showed the potential use of Ys as a source of antioxidant principle.

In this study, experimental groups were fed by different amount of Ys powder. Antioxidant markers GSH and MDA were evaluated for basal oxidative stress. The results of the present study have demonstrated that 100 and 200 ppm of Ys powder supplementation has affected the MDA concentration in blood and kidney of the rats. But Ys powder did not affect liver MDA level. The results in the present study also showed that the GSH concentrations significantly increased in kidney by adding 200 ppm of Ys powders in diets. These results suggested that Ys supplementation exhibited direct antioxidant properties by reducing basal MDA formation and protective antioxidant effect. Furthermore this protective effect could be oving to the resveratrol content of Ys. The decrease in MDA concentrations could be due to the Ys ability to scavenge secondary reactive radicals and this mechanism could play a preventive role aganist the formation of superoxide and hydrogen peroxide during the normal metabolic activity.

According to the findings of this study, 100 and 200 ppm supplementation of Ys powder to the diets of rats, decrease the blood and kidney MDA levels and increase the kidney GSH concentrations. It is thought that Ys can be used effectively as an antioxidant suplement. Moreover, the usage of the plant, did not affect the liver MDA, GSH as well as blood GSH levels. Therefore, due to such reason, new studies should be carried out on this subject towards understanding the effects of Ys powder supplementation into diets of animals.

CONCLUSION

Yucca schidegera may be considered as an appropriate potential food stuff and a phenolics supplement of good nutritonal properties of animals. The Ys powder would considerably reduce oxidative stress and maintain life span of animals.

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