International Journal of Biotechnology Applications
ISSN: 0975–2943 & E-ISSN: 0975–9123, Vol. 2, Issue 2, 2010, pp-33-40

Antibiotic susceptibility and antioxidant activity of *Staphylococcus aureus* pigment staphyloxanthin on carbon tetrachloride (ccl_4) induced stress in swiss albino mice

Kurjogi M. M., Sanakal R. D., Kaliwal B. B. *
*P.G. Department of Studies in Biotechnology and Microbiology, Karnatak University, Dharwad, 580003, India, b_kaliwal@yahoo.com

Abstract-The present investigation was under taken to select a suitable antibiotic for the treatment of bovine mastitis by conducting antibiotic sensitivity test with different antibiotics and to evaluate the antioxidant property of pigment staphyloxanthin produced by *S. aureus* against Carbon tetrachloride (CCl_4) induced stress in albino mice. In this study a total of 20 samples were screened and out of which 12 samples confirmed *Staphylococcus aureus* were obtained. The confirmed strains of *Staphylococcus aureus* were subjected to haemolytic activity. α, β and non-haemolytic activities were**,** 33.33%, 50% and 16.67% observed respectively among the isolates of *Staphylococcus aureus*. Antibiotic susceptibility of *staphylococcus aureus* against 5 antibiotics was tested by using standard antibiotic discs. Ceftriaxone was found to be the most effective drug and also mice is used as a model to study the antioxidant property of *staphylococcus aureus* pigment against a stress induced by CCl_4 in mice. Results showed that animals treated with 1ml/kg body weight of CCl_4 for 2 days caused a marked rise in the level of thiobarbituric acid reactive substances (TBARS) and decreased glutathione Reduced glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione-S-transferase (GST) levels in the liver, kidney and testis tissue homogenates of CCl_4 treated mice. Graded doses of *Staphylococcus aureus* pigment Staphyloxanthin successfully prevented the alterations of these effects in the experimental mice.
Key words: *Staphylococcus aureus.* Pigment. Antioxidant. CCl_4 carotinoid, staphyaloxanthin thiobarbituric acid reactive substances, glutathione Reduced glutathione

Introduction

It has been shown *in vitro* and *in vivo* that the carotenoid pigment protects the wild type *S. aureus* from hydrogen peroxide and singlet oxygen oxidation; as well as neutrophilic or phagocytic killing [28]. Furthermore, β-carotene has been shown to protect cells and organisms against oxidation by having the ability to act as a chain-breaking radical scavenger, because they are highly reactive to singlet oxygen and other radical species including those that are produced during oxidative stress [11]. It has been suggested that bacterial carotenoids such as those expressed by *S. aureus* could serve a protective function against these defense molecules [28].

Carbon tetrachloride is an organic solvent which is well known hepatotoxin, induce oxidative stress and causes tissue damage. The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against such hepatic damage, search for crude drugs of plant and microbial origin with antioxidant activity has become a central focus for study of hepatoprotection today. In the present study the carbon tetrachloride is used to induce oxidative stress in swiss albino mice therefore the present investigation was under taken to evaluate the antioxidant property of pigment staphyloxanthin produced by *S. aureus* against CCl_4 induced stress in albino mice.

Materials and methods

Collection of samples

Milk samples were collected hygienically and analyzed for presence of bacteria as described by Honkanen-Buzalski [21]. The colony characteristics of the isolated colonies were studied as per the ones which matched with the colony characteristics of *S. aureus* was selected for further study.

Phenotypic characterization

The isolated organisms have been identified and studied by carrying out Gram's staining, microscopic observations and biochemical tests for catalase, Methyl red – Vogus Proskauer test, mannitol fermentation coagulase and gelatin tests. The isolates have been identified According to the standard methods. The confirmed cultures of *S. aureus* were streaked on blood agar plates to study its hemolytic pattern.

Antibacterial susceptibility test

Antibiotic susceptibility screening was done as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). Kirby-Bauer's disc diffusion technique was adapted for antibiogram. The antibiotic discs and Mueller- Hinton Agar were procured from Hi-Media, Mumbai. The plates were prepared as per the manufacturer's instructions and checked for sterility by incubating the plates overnight at 37℃.

Extraction of pigment (staphyloxanthin)

Extraction of staphyloxanthin pigment was done by following the method of [7]. Nutrient broth culture was centrifuged at 10,000 rpm for 15 min and the supernatant was extracted with ethyl acetate. The pigment from the cell pellet

was extracted with acetone and the extraction was centrifuged at 10000 rpm for 15 minutes.The white pallets were discarded. Ethyl acetate fraction and acetone fraction was mixed and dried with anhydrous sodium sulphate and the extract was evaporated, residue is collected and stored in refrigerated condition until further use.

Animals

Ninety days old healthy male swiss albino mice weighing 25 – 30 gms were used for the experiment. The mice were maintained aseptically in laboratory condition (25 ± 2ºC and with 12 hrs light/dark cycle) and unlimited access to pellet diet "Gold Mohar" (Hindustan Lever Ltd., Mumbai) and water throughout the study in the animal house, P.G. Department of Studies in Zoology, Karnatak University, Dharwad. Daily body weights were recorded throughout the experiment.

Treatment

Carbon tetrachloride (CCl_4 99%) used in experiment was organic solvent procured from RANKEM India Pvt., Ltd. The CCl_4 was given orally in olive oil vehicle (1:1 v/v) according to the body weight of mice i.e., 1ml/kg body weight/day for 2 days to respective groups.

The Pigment extract used in the experiment is made into solution by olive oil and the graded dose of pigment extract of 0.05 ml and 0.1 ml was administrated orally to mice of respective groups. The experiment was designed to determine the effect of pigment extract of *staphylococcus aureus* over CCl_4 on weight, protein content and oxidative stress parameters in respective organs of albino mice being tested**.**

Animals were divided into 6 groups of 10 mice each, as follows

Group A mice were treated with distilled water in quantities equivalent to the volume of CCl_4 administrated orally which is used as control. The mice in group B were treated with 1 ml/ kg body weight of CCl_4, the mice in group C were treated with CCl_4 for two days and were additionally treated with pigment extract of 0.05 ml for 4 days. The mice in group D were treated with 1 ml/ kg body weight of CCl_4 for two days and were additionally treated with pigment extract of 0.1 ml for 4 days. The mice in group E were treated with 1 ml/ kg body weight of CCl_4 for two days and were additionally treated with pigment extract of 0.05 ml for 6 days.The mice in group F were treated with 1 ml/ kg body weight of CCl_4 for two days and were additionally treated with pigment extract of 0.1ml for 6 days.

All the experimental animals were autopsied by cervical dislocation after 24 hrs of the terminal exposure. The organs viz. liver, kidney and testes were dissected out weighed to the nearest milligrams in digital weighing balance (vibra) and were also used for estimations of oxidative stress parameters in the albino mice.

Preparation of tissue homogenate

The tissues were thawed and homogenized in 10% w/v ice-cold 0.05 M potassium phosphate buffer (pH 7.4). The homogenate (0.2 ml) was used for TBARS estimation. The homogenate (1.0 ml) was mixed with 10% trichloroacetic acid (TCA) and centrifuged for tissue GSH estimation. The remaining homogenate was centrifuged at 40,000 × g for 60 min and the supernatant was used for estimations of superoxide dismutase (SOD) and catalase (CAT) activity.

Estimation of Protein

Protein content of different tissues in the present study was quantified by [26] method.

Assay of enzymes and non-enzymatic antioxidant in the liver, kidney and testes homogenate

Liver SOD activity was assayed as per the method [23], catalase by [3], GST by [20], GSH by [12] and TBARS by[13].

Statistical analysis

The results obtained were analyzed statistically by ANOVA following Dunnett`s test ($p \leq 0.05$). for significance between control and treated groups.

Results

Antibiotic susceptibility of *staphylococcus aureus*

All colonies of *Staphylococcus* have shown positive results for catalase, mannitol fermentation, gelatin hydrolysis, MR-VP and coagulase tests. 12 strains of *Staphylococcus* were confirmed as *Staphylococcus aureus* based on conventional methods.

The confirmed strains of *Staphylococcus aureus* were subjected to haemolytic activity test. The haemolytic activities of α, β and non-haemolytic were**,** 33.33%, 50% and 16.67% respectively was observed among the isolates of *Staphylococcus aureus*.

The results of the present study suggests that the antibiotic susceptibility testing of *Staphylococcus aureus* to various antibiotics revealed higher resistance to ciprofloxacin followed by ampicillin and the lower resistance was shown in ceftriaxone, methicillin and vancomycin.

Antioxidant activity of *staphylococcus aureus* pigment staphyloxanthin

The data obtained in the present investigation revealed that the level of protein contents in liver, kidney and testes was decreased in the mice treated with CCl_4. there was a gradual increase in the level of protein in liver, kidney and testes in the mice treated with SP along with CCl_4, (table.1)

Oxidative stress parameters of organs in mice after exposure to carbon tetrachloride (CCl_4) and staphyloxanthin pigment (SP)

Effect on GSH level of liver, kidney and testes for all experimental groups are shown in table 2, 3 and 4 respectively. In the present study CCl_4 treatment caused significant decrease in GSH level in liver, kidney and testes tissue. Whereas, in the mice receiving SP along with CCl_4 showed gradual increase in the level of GSH.

Effect on TBARS level of liver, kidney and testes for all experimental groups are shown in table 2, 3 and 4 respectively. In the present study CCl_4 treatment caused significant increase in TBARS level of liver and kidney tissue, whereas CCl_4 treatment along with SP showed decrease in the level of TBARS.

The activity of SOD, CAT and GST in liver, kidney and testes tissue homogenates are shown in table 2, 3 and 4 respectively. The activity of SOD, CAT and GST in liver, kidney and testes tissue homogenates of CCl_4 treated mice was considerably reduced, whereas, in the mice treated SP along with CCl_4 showed an increase in SOD, CAT and GST activity.

Discussion

The *in vitro* antibiotic susceptibility of a pathogen may not be necessarily indicate successfulness. But antibiotic resistance can be interpreted as high probability failure of treatment. Absence of prophylactic agents and chemotherapy continues to play a major role in therapeutic management of treatment. The emergence of antimicrobial resistance among pathogens that affects animal health is of growing concern in veterinary medicine. Antimicrobial resistant of pathogens in animals have been considered as a potential health risk for humans from possible pathogens. The above findings clearly indicate that intermittent changing pattern of antibiotic susceptibility against *S.aureus* may be ascribed to the extent of different antibiotics to be used from locality to locality. From our study, it is clear that methicillin is the most sensitive chemotherapeutic agent. Therefore it is compulsory that antibiogram investigation is to be made from time to time in a locality to be on the lookout for the most effective antibiotic against the existing mastitogens i.e., bacteria. The proportion of isolates that were resistant to the antimicrobial agents tested were within the range of other findings reported from Germany using the same breakpoints [48, 49].

Antioxidant activity of *staphylococcus aureus* pigment staphyloxanthin

The results indicate that SP could be used as effective protector against CCl_4 induced stress in mice. Evidence suggests that reactive metabolite of CCl_4 is trichloromethyl radical ($-CCl_3$) which is known to be formed from the metabolic conversion of CCl_4 by cytochrome $P\text{-}_{450}$. As O_2 tension rises, a greater fraction of $-CCl_3$ present in the system reacts very rapidly with O_2 and high reactive free radical, $-CCl_3OO$ is generated from $-CCl_3$ [34]. These free radicals initiate the peroxidation of cell membrane poly-unsaturated fatty acids (PUFA) [38] and covalently bind to microsomal lipids and proteins [47]. The obtained results coroborated with other studies [36] who found that xenobiotic compounds decreased total serum proteins in treated animals. They also reported that albumin (A) content was decreased while the globulins (G) were increased in the same. It has been reported [41] a decrease in protein content of blood of xenobiotic intoxicated animals (fish), and suggested that the decline in protein level indicates the physiological adaptability to compensate for xenobiotic compound stress in fish. To overcome the stress, the animals use more energy, which leads to stimulation of protein catabolism. Therefore, in present findings the decrease in the protein content may be due to the intoxication of free radicals. This can be rectified by the treatment of SP through its antioxidant property.

Oxidative stress parameters of organs in mice after exposure to carbon tetrachloride (CCl_4) and staphyloxanthin pigment (SP)

The result indicates that SP acts as a protective agent against CCl_4 toxicity. It has been reported that treatment of CCl_4 reduces the level of GSH in liver and kidney. Treatment with aqueous extract of *Terminalia arjuna* and vitamin E along with CCl_4 showed recovery in the level of GSH in mice [37]. It has been also suggested that GST binds to liophilic compounds and acts as an enzyme for GSH conjugation reactions [6]. Decrease in GSH level during CCl_4 toxicity might be due to the decreased availability of GSH that resulted during the enhanced lipid peroxidation. It has been reported that administration of Withania root extracts in mice increase the activity of mainly phase II enzymes such as SOD, CAT, GST and GSH level [8]. It has been reported that exclusive exposure of animals to man made chemicals triggered a decrease in the GSH content in rat liver and kidney only in the first period after intoxication (up to the 24th h). However, a greater decrease was observed after mixed intoxication. Corresponding results were obtained earlier [51] who used a concentrate of technical grade of xenobiotic compounds. It has been reported that in transgenic mice may be explained by the rapid depletion of GSH in response to xenobiotic exposure [47]. This GSH depletion may result from participation of GSTs in the removal and reduction of (hydro) peroxides at the expense of GSH utilization. In the present study, it has been observed that administration of SP along with CCl_4 could prevent the CCl_4 induced stress. The decreased level of GSH in CCl_4 treated mice may be due to the decreased availability of GSH that resulted during the enhanced lipid peroxidation. GSTs Participate in the removal and reduction of (hydro) peroxides at the expense of GSH utilization. The increased level of GSH in SP along with CCl_4 treated mice may be due to the increase

in the activity of mainly phase II enzymes that helps in the detoxification.

It has been suggested that treatment with CCl_4 increased the level of TBARS in liver and kidney and treatment with aqueous extract of *Terminalia Arjuna* and vitamin E along with CCl_4 showed recovery in the level of TBARS in mice [38]. It has been reported that metabolism of CCl_4 by cytochrome P_{450} initiate free radical – mediated lipid peroxidation leading to accumulation of lipid peroxidation products that cause tissue injury. These radicals are capable of initiating a chain of lipid peroxidation reaction by binding covalently to microsomal lipids protein which can cause change in biological membranes, resulting in severe tissue damage. The evidence suggests that the modulatory effect of the plant extracts on the detoxification enzymes and reduction of lipid peroxidation in tissue. Xenobiotic chemicals have also been reported to have high mammalian toxicity, and the main target organs are brain, liver, skeletal muscles, and heart [20]. It has been previously reported that chronic exposure to these chemicals is responsible for the oxidative injury leading to perturbations in membrane structure and functions [26]. It was also observed the increase in lipid peroxidation in skeletal muscles after exposure to these chemicals and has attributed this increase due to increased formation of reactive oxygen and nitrogen species [32]. Several studies with liver, brain, kidney and testes indicate that many of man made chemicals cause oxidative stress [40, 23, 41, 1, 16, 45]. Lipid peroxidation has been shown to increase in plasma and some tissues in xenobiotic intoxication [36, 4, 16, 5]. Studies indicate that pesticide intoxication produce oxidative stress by the generation of free radicals and induce tissue lipid peroxidation in mammals and other organisms [11]. The present study showed that treatment of CCl_4 increased the level of TBARS may be due to the generation of free radicals and the treatment of SP along with CCl_4 decreased the level of TBARS. This indicates that SP exerted a therapeutic effect on CCl_4 induced stress in mice, possibly through its antioxidant action and may be due to the modulatory effects of SP.

That treatment with CCl_4 reduces the level of catalase in liver and kidney. The treatment of aqueous extract of *Terminalia Arjuna* and vitamin E showed recovery in the level of SOD, CAT and GST in mice [38]. It has been reported that SOD, CAT and GST constitute a mutually supportive team of defense against ROS. The decreased activity of SOD in liver and kidney in CCl_4 treated mice may be due to the enhanced lipid peroxidation or inactivation of the antioxidative enzymes. This would cause an increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation. Evidences suggest that body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT and GST. GPx converts toxic lipid hydroperoxides and using reducing equivalents generated by G6PDH [17]. Investigations [17] indicated that mammalians have a good defense mechanism for lipid peroxidation because it can increase the hepatic CAT activity when needed. CAT is generally localized in peroxisomes and therefore, its role in the other parts of the cell is limited. In particular, H_2O_2 at low concentration is destroyed by this enzyme [26, 2, 35]. Indeed, GST participates in pollutant detoxification by adding a GSH-group to xenobiotics or their metabolites. Hence they become more waters soluble and, thus, excreted more easily [34]. In the present study, decline in the level of antioxidant enzymes like SOD, CAT and GST observed in CCl_4 treated mice is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage. The increased level of SOD, CAT and GST may be due to the increase in the activity of mainly phase II enzymes that helps in detoxification. The decrease in concentration of SOD, CAT and GST in mice treated with CCl_4 may be due to enhanced lipid peroxidation or inactivation of the antioxidant enzymes and increase in the concentration of these constituents in tissues of mice treated with SP along with CCl_4 indicate antioxidant effect of SP. Further investigation is essential to fully characterize the responsible active principles present in the *S.aureus* pigment extract and understand its possible mechanism of action on CCl_4 induced toxicity in mice.

Conclusion

The present study demonstrated that the existence of alarming level of resistance of frequently isolated mastitis bacteria to commonly used antimicrobial agents in the farms where study was undertaken. Therefore, it is very important to implement a systematic application of an in vitro antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections. In this study it has also been demonstrated that Staphylococcus aureus, which contain a yellow pigment called staphyloxanthin belonging to the precursor of beta carotene may act as an antioxidant which prevents CCl_4 induced toxicity in liver, kidney and testis in mice.

Acknowledgment

The authors are grateful to the Post Graduate Department of Studies in Microbiology and Biotechnology, Karnatak University Dharwad for providing the necessary facilities.

Reference

[1] Abdollahi M. Ranjabar A. Shadina S. Mikfar S. and Razaice A. (2004) *A Review. Med. Sci. Monit,* 10, 144-147.

[2] AdalÝ M. Ünal-Erden M. AkalÝn A. and Efe B. (1999) *Clin. Biochem,* 32, 363-367.
[3] Aebi H. (1974) *Meth. Enzymol,* 105, 121–6.
[4] Akhtar M.H. Mahadevan S. Paquet A. (1994) *J. Environ. Sci Hlth,* 29, 369-394.
[5] Aldana L. Tsutsumi V. Craigmill A. Silveria M.I. Mejia E.G. (2001) *Toxicol. Lett,* 125, 107-116.
[6] Anandan R. Deepa Rekha R. and Devaki T. (1999) *Curr Sci,* 76, 1543-1545.
[7] Anurada V. Giri Nandini Anandkumar Geetha Muthukumaran and Gautam Pennathur (2004) *BMC. Microbiology,* 4(11), 4-11.
[8] Bandhuvula Padmavathi. Pramod C. Rath Araga Ramesha Rao. and Rana Pratap Singh. (2005) *Oxford University Press,* 10.1093/ecam/neh064.
[9] Bonaventura J. Schroeder W.A. Fang S. (1972) *Arch Biochem. Biophys,* 150, 606-617.
[10] Cereser C. Boget S. Parvaz P. and Revol A. (2001) *Toxicology,* 163, 62–153.
[11] Çömelekoglu Ü. Mazmanci B. and Arpaci A. (2000) *Turk. J. Biol,* 24, 483-488.
[12] El-Agamey A. Lowe G.M. McGarvey D.J. Mortensen A. Phillip D.M. Truscott T.G. and Young A.J. (2004) *Arch. Biochem. Biophys,* 430, 37-48.
[13] Ellman G.L. (1959) *Arch Biochem. Biophys,* 82, 70-77.
[14] Esterbauer H. Cheesman K.H. (1990) *Methods Enzymol,* 186, 407-421.
[15] Fang F.C. (2004) *Nat. Rev. Microbiol,* 2, 820-832.
[16] Faraone-Mennella M.R. Malanga M. and Petrella A. (2003) Determination of F Monit, 9, 23–30.
[17] Frei B.L. England and Ames B.N. (1989) *Biol. Med,* 31, 745-753.
[18] Gabbianelli R. Falcioni G. Nasuti C. Cantalamessa F. (2002) *Toxicology,* 175, 91-101.
[19] Gale G.R. Smith A.B. Atkins L.M. Walker E.M. and Gadsen R.H. (1971) *Toxicol. Appl. Pharmacol,* 18, 426-441.
[20] Gupta A. Nigam D. Shukla G.S. Agarwal A.K. (1999) *J. Appl. Toxicol,* 19, 67-72.
[21] Gupta R. C. (1994) *J. Toxicol. Environ. Health,* 43, 383–418.
[22] Habig W.H. Jakoby W.B. (1974) *J. Biol. Chem,* 249, 7130-7139.
[23] Honknen-Buzalski T. (1995) *NMC Inc., Madison W.I,* 111-114.
[24] Junqueira V.B. Koch O.R. and Arisi A.C. (1997) *Toxicol,* 117, 199–205.
[25] Kakkar P. Das B. Viswanathan P.N. (1984) *Ind. J. Biochem. Biophys,* 21, 130-132.
[26] Kale M. Rathore N. John S. and Bhatnagar D. (1999) *Toxicol. Lett*, 105**,** 197-205.
[27] Kamboj A. Kiran R. and Sandhir R. (2006) *Mol. Cell. Biochem,* 286, 107–114.
[28] Lowry H. Rosebrough N.I. For A.L. Randall R.I. (1951) *J. Biol. Chem,* 193, 265-275.
[29] Lightner J.K. Miller G.Y. Hueston W.D. and Dorn C.R. (1988) *J. American Vet. Med. Assoc*, 192, 1410-3.
[30] Liu G.Y. Doran K.S. Lawrence T. Turkson N. Puliti M. Tissi L. and Nizet V. (2004) *Proc. Natl. Acad. Sci. USA,* 101, 14491-14496.
[31] Łukaszewicz-hussain A. and Moniuszko-jakoniuk J. (2003) *Pol. J. Environ. Stud,* 12, 417- 423.
[32] Michelangeli F. Robson M.J. East J.M. and Lee A.G. (1990) *Biochim. Biophys. Acta,* 1028, 49-57
[33] Milotovic D. Gupta R.C. Dekundy A. Montine T.J. and Dettbarn W.D. (2005) *Toxicology,* 208, 13–24.
[34] Moorhouse K.G. and Casida J.E. (1992) *Pestic. Biochem.Physiol,* 44, 83–90.
[35] Ner N. .zcan Oru E. CanlÝ M. Sevgiler Y. (2001) *Bull. Environ. Contam. Toxicol,* 67, 657- 664.
[36] Packer J.E. Slater T.F. and Willson R.L. (1979) *Nature (London),* 278, 737–738.
[37] Parker C.M. Patterson D.R. Van Gelder G.A. Gordon E.B. Valerio MG and Hall W.C. (1984) *J. Toxicol. Environ. Health,* 13, 83-97.
[38] Prabhakaran S. Shameem F. and Devi K.S. (1993) *Veterinary and Human Toxicolgy,* 35, 429-433.
[39] Prasenjit Manna. Mahua Sinha. and Parames C. Sil. (2006) *BMC Complementary and Alternative Medicine,* 6, 33.
[40] Recknagel R.O. Glendek E.A. jr Dolazk J.A. and Waller R.L. (1989) *Pharmacol. Ther,* 43, 139-154.
[41] Sahoo A. Samanta L. and Chainy G.B. (2000) *Arch Environ Contam. Toxicol,* 39, 7–12.
[42] Samanta L. Sahoo A. and Chainy G.B. (1999) *Arch Toxicol,* 73, 96–107.
[43] Sancho E. Ferrando M.D. Fernandez C. and Andreu E. (1998) *Ecotoxicol. Environ. Saf,* 41, 168-175.
[44] Shakoor A. (2005) *PhD Dissertation, Deptt. Vet. Clinical Medicine and Surgery, Univ. Agri., Faisalabad, Pakistan*.
[45] Sharma Y. Bashir S. Irshad M. Nag T.C. and Dogra T.D. (2005) *Toxicology,* 215, 173-181.

[46] Simon-Giavarotti K.A. Giavarotti L. and Gomes L.F. (2002) *Free. Rad. Res,* 36, 1033–39.
[47] Spodniewska A. and Zasadowski A. (2008) *ACTA VET, BRNO,* **77**, 355–362.
[48] Suntres Z.E. (2002) *Toxicology,* 180, 65–77.
[49] Tam W.M. Fong D. Woo B. Prasongwatana V. and Boyde T.R. (1984) *Chem. Biol. Interact,* 50, 361-363.
[50] Trolldenier H. (1999) *Zur Resistenzproblematik in der Veterina rmedizin- U bersicht aus bundesweit erfassten Daten. Tierarztl. Prax,* 27, 317–323.
[51] Wallmann J.K. Schroter L.H. Wieler and Kroker R. (2003) *Int. J. Antimicrob. Agents,* 22(4), 420-428.
[52] Wysocki A. and Zasadowski A. (2005) *Acta. Toxicol,* 13, 67-76.

Table 1- Effect on organs protein contents in mice after exposure to carbon tetrachloride (CCl_4) and staphyloxanthin pigment (SP)

Group	Treatment	protein contents (µg / mg wet weight of tissue)		
		liver	kidney	testes
A	control	245.40 ± 3.06	261.70 ± 6.70	192.06 ± 4.40
B	CCl_4	167.75 ± 5.70*	161.01 ± 6.89*	159.11±2.5*
C	CCl_4 + SP 0.05 ml for 4 days	196.52±4.1*	186.32±5.32*	165.23 ±2.61*
D	CCl_4 + SP 0.1 ml for 4 days	209.21±4.11	206.44±5.62	171.54 ±3.12
E	CCl_4 + SP 0.05 ml for 6 days	231.57±3.61	228.56±2.48	183.22 ±4.01
F	CCl_4 + SP 0.05 ml for 6days	249.62±3.46	257.24±2.33	190.20 ±4.00

Table 2- Oxidative stress parameters of liver in mice after exposure to carbon tetrachloride (CCl_4) and staphyloxanthin Pigment (SP)

grou	Treatment	Antioxidant	Oxidative stress byproducts	Oxidative stress enzymes		
		GSH [a]	TBARS [b]	Catalase [c]	SOD [d]	GST [e]
A	Control	10.00 ± 0.20	0.180 ± 0.015	150.0 ± 0.002	38.00 ± 0.0.070	4.500 ± 0.04
B	CCl_4	6.45 ± 0.165*	0.618 ± 0.003*	56.20 ± 1.544*	19.75 ± 0.263*	2.130 ± 0.024*
C	CCl_4 + SP 0.05 ml for 4 days	8.22 ± 0.179*	0.422 ± 0.004*	82.47 ± 1.289*	33.68 ± 0.267*	3.050 ± 0.064*
D	CCl_4 + SP 0.1 ml for 4 days	9.47 ± 0.226	0.267 ± 0.004*	104.5 ± 2.47*	36.94 ± 0.073*	3.405 ± 0.411*
E	CCl_4 + SP 0.05 ml for 6 days	11.18 ± 0.208*	0.222 ± 0.004*	126.50 ± 1.70*	38.55 ± 0.212	3.805 ± 0.411*
F	CCl_4 + SP 0.1 ml for 6 days	13.00 ± 0.187*	0.176 ± 0.004*	146.40 ± 0.355	40.13 ± 0.319*	4.235 ± 0.079*

a GSH u mol/ mg protein **b** TBARS u mol /mg protein **c** CAT µmole/min/mg protein H_2O_2
d SOD u/mg protein **e** GST µmole/min/mg protein
Values are mean± SEM of 10 animals * Significant $P \leq 0.05$ compared to control

Table 3- Oxidative stress parameters of kidney in mice after exposure to carbon tetrachloride (CCl_4) and staphyloxanthin pigment (SP)

Group	Treatment	Antioxidant	Oxidative stress byproducts	Oxidative stress enzymes		
		GSH [a]	TBARS [b]	Catalase [c]	SOD [d]	GST [e]
A	Control	7.80 ± 0.25	0.195 ± 0.01	33.00 ± 0.201	52.00 ± 0.80	0.85 ± 0.004
B	CCl_4	4.53 ± 0.19*	0.616 ± 0.003*	18.11 ± 0.133*	37.07 ± 0.75*	0.44 ± 0.006*
C	CCl_4 + SP 0.05 ml for 4 days	6.010 ± 0.07*	0.425 ± 0.002*	21.92 ± 0.165*	41.30 ± 0.85*	0.52 ± 0.004*
D	CCl_4 + SP 0.1 ml for 4 days	6.97 ± 0.055*	0.260 ± 0.00*	24.58 ± 0.203*	47.35 ± 0.50*	0.65 ± 0.012*
E	CCl_4 + SP 0.05 ml for 6 days	7.62 ± 0.15	0.230 ± 0.00*	27.67 ± 0.247*	50.22 ± 0.64	0.81 ± 0.005*
F	CCl_4 + SP 0.1 ml for 6 days	8.24 ± 0.10	0.212 ± 0.002*	31.40 ± 0.219*	54.95 ± 0.10*	0.83 ± 0.001

a GSH u mol/ mg protein **b** TBARS u mol /mg protein **c** CAT µmole/min/mg protein H_2O_2
d SOD u/mg protein **e** GST µmole/min/mg protein
Values are mean± SEM of 10 animals * Significant $P \leq 0.05$ compared to control

Table 4- Oxidative stress parameters of testes in mice after exposure to carbon tetrachloride (CCl_4) and staphyloxanthin pigment (SP)

gro	Treatment	Antioxidant	Oxidative stress byproducts	Oxidative stress enzymes		
		GSH [a]	TBARS [b]	Catalase [c]	SOD [d]	GST [e]
A	control	8.80 ± 0.012	0.210 ± 0.0027	42.00 ± 0.01	33.00 ± 0.152	0.685 ± 0.003
B	CCl_4 25 µl	8.08 ± 0.031*	0.205 ± 0.0004	40.11 ± 0.11*	28.72 ± 0.262*	0.672 ± 0.0020*
C	CCl_4 + SP 0.05 ml for 4 days	8.30 ± 0.019*	0.207 ± 0.002	41.13 ± 0.068*	30.75 ± 0.351*	0.674 ± 0.0021*
D	CCl_4 + SP 0.1 ml for 4 days	8.50 ± 0.027*	0.200 ± 0.00*	42.29 ± 0.207	32.77 ± 0.417	0.683 ± 0.0017
E	CCl_4 + SP 0.05 ml for 6 days	8.64 ± 0.016*	0.187 ± 0.002*	44.62 ± 0.196*	34.13 ± 0.158*	0.691 ± 0.0007*
F	CCl_4 + SP 0.1 ml for 6 days	8.87 ± 0.021	0.179 ± 0.0004*	46.47 ± 0.218*	35.47 ± 0.188*	0.696 ± 0.0007*

a GSH u mol/ mg protein **b** TBARS u mol /mg protein **c** CAT µmole/min/mg protein H_2O_2
d SOD u/mg protein **e** GST µmole/min/mg protein
Values are mean± SEM of 10 animals * Significant $P \leq 0.05$ compared to control