Antioxidant and protective effects of Phytocee™ against carbon tetrachloride-induced oxidative stress

Joshua Allan Joseph, Uma Radhakrishnan¹, Sridhar Mutyala², Krishnagouda Shankar Goudar², Usha Parackal Thachappully Ayyappan, Amit Agarwal²

Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, ¹Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala ²Department of Pharmacology and Toxicology, R and D Center, Natural Remedies, Bengaluru, Karnataka, India

Address for correspondence: Dr. Joshua Allan Joseph, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur - 680 651, Kerala, India. E-mail: jjoshuaallan@gmail.com

Abstract

Background: This study evaluated the antioxidant potential of a polyherbal formulation (Phytocee™) in the rodent model. Materials and Methods: Four groups of rats (n = 6) were pretreated with Vitamin C (20 mg/kg) or Phytocee™ (20, 100, and 200 mg/kg), respectively for 10 days. Oxidative stress in rat liver was induced by administration of carbon tetrachloride (CCl4) at 2 ml/kg as a single dose orally to all groups except the vehicle control group. After 24 h of administration of CCl4, hepatic levels of malondialdehyde (MDA), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), hepatic superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) levels were evaluated. Results: Phytocee™ administered groups at all the dose levels significantly reduced the hepatic MDA, serum ALT and AST levels with a marked increase in hepatic SOD and catalase as compared with CCl4 treated group. Conclusion: The findings suggest that Phytocee™ markedly reversed the effects of CCl4 induced oxidative stress and can be used as an antioxidant feed supplement.

Key words: Antioxidant, carbon tetrachloride, malondialdehyde, oxidative stress, Phytocee™

INTRODUCTION

Natural antioxidant supplementation to combat stress susceptibility has been an area of substantial interest to researchers and livestock producers. Livestock’s experiencing stress show several metabolic and hormonal responses and one such common effect of stress is an increase in the metabolic rate.[1] Higher metabolic rate leads to increased free radical and reactive oxygen species production.[2] In general, inherent antioxidant defense systems deactivate the free radicals and protect the organisms from radical toxicity.[3] However, when there is excessive production of free radicals, imbalance between oxidants and antioxidant defense system (enzymatic and nonenzymatic) provokes oxidative stress.[4] The inherent mechanisms may be inadequate in stressful conditions causing the oxidant-antioxidant equilibrium to shift toward a pro-oxidative status.[5] A great body of evidence suggests that oxidative stress and the resulting lipid peroxidation (LPO) leads to oxidative damage and affects performance.[1,6] Consequently, exogenous antioxidant supplementation in diet would prevent or ameliorate the oxidation process and bring about homeostasis between oxidants and antioxidants.

Several herbs and herbal formulations have gained considerable importance in the vital healthcare supplements.[7-11] Although scientific evidence is available for antioxidant activity of several individual plants, the
polyherbal formulation Phytocee™ (M/s Natural Remedies, Bengaluru, India) containing Emblica officinalis, Ocimum sanctum and Withania somnifera as principal ingredients was not scientifically validated for its efficacy in vivo. This study was designed to evaluate the antioxidant activity of Phytocee™ using carbon tetrachloride-induced liver oxidative stress model in Wistar rats.

MATERIALS AND METHODS

Animals
Male albino Wistar rats bred at Central Animal Facility, Research and Development Center, Natural Remedies, Bengaluru were used. Animals were housed under standard laboratory conditions (12 h/12 h light/dark cycle at 25°C ± 2°C and 30-70% relative humidity) and provided free access to pelleted rodent feed (M/s Amrut Laboratory Animal Feeds, Pranav Agro Industries Limited, Sangli, India) and ultraviolet purified and filtered water ad libitum. This study was approved by Institutional Animal Ethics Committee (IAEC/ PCL/04/02.09).

Chemicals and reagents
Vitamin C purified/ascorbic acid (Merck Specialities Private Limited, Mumbai, India), carbon tetrachloride (CCl₄) (Rankem Fine Chemicals Limited, New Delhi, India), Refined olive oil (SOS Cuetara, S. A, Madrid, Spain) were obtained. Other chemicals used were 2-thiobarbituric acid, 5, 5'-dithio bis (2-nitro-benzoic acid) (Sigma — Aldrich Co., USA), bovine albumin fraction-V, ethylene diamine tetra acetic acid, di-sodium salt, Copper (II) sulfate, pentahydrate, potassium sodium tartrate, tetrahydrate, triton X-100 (HiMedia Laboratories Pvt. Ltd., Mumbai, India), pyrogallol, potassium dihydrogen orthophosphate (Qualigens Fine Chemicals, Mumbai India), tris buffer, hydrogen peroxide solution 30%, potassium dihydrogen phosphate, di-sodium hydrogen orthophosphate, hydrochloric acid, trichloro acetic acid, and methanol (Ranbaxy Fine Chemicals Limited, New Delhi, India). All other chemicals and reagents used were of analytical grade.

Plant materials
Phytocee™ is a novel polyherbal formulation developed by M/s. Natural Remedies, Bengaluru, India, containing E. officinalis fruits (70% w/w), O. sanctum whole plant (20% w/w), and W. somnifera roots (10% w/w).[12]

Experimental protocol
Male rats (n = 36) were randomly allotted to six groups each consisting of six animals. Group I was administered with vehicle control (demineralized water 10 ml/kg), Group II served as a negative control (CCl₄ with olive oil in 1:1 ratio). The remaining four groups were administered orally with Vitamin C (20 mg/kg), or Phytocee™ (20, 100, 200 mg/kg). Vehicle, Vitamin C and Phytocee™ were administered for 10 days to the respective groups and all animals except in vehicle control group were challenged with carbon tetrachloride (1:1 in olive oil).[13] The animals were anesthetized 24 h after CCl₄ administration, blood was drawn and serum was separated for biochemical analysis. Animals were euthanized; liver was excised, blotted and processed for the biochemical assays.

Biochemical analysis
Lipid peroxidation levels in liver homogenates, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST),[15] hepatic superoxide dismutase (SOD),[16] catalase activities,[17] and reduced glutathione (GSH)[18] levels were estimated by previously described methods.

Statistical analysis
Data are represented as mean ± standard error of the mean and were analyzed using one-way ANOVA followed by Bonferroni method as post-hoc test. In case of heterogeneous data after transformation, Dunnett T3 method was used. Statistical significance was set at P ≤ 0.05.

RESULTS
The mean hepatic malondialdehyde (MDA) levels are shown in Figure 1. The CCl₄ control group exhibited a significant increase in MDA levels as compared with the vehicle control. However, groups pretreated with Phytocee™ at all the dose levels showed significant (P ≤ 0.05) decrease in the hepatic MDA levels as compared with the CCl₄ control group.

Mean serum ALT and AST levels, which are markers for hepatic tissue damage are represented in Figure 2. The CCl₄ control group exhibited a significant increase in serum ALT and AST levels when compared with the vehicle control group. The groups that were pretreated with Phytocee™ at all dose levels showed a significant decrease in the activities of marker enzymes levels compared to CCl₄ group (P ≤ 0.05).

The mean values of hepatic antioxidant defenses, enzymatic (SOD and catalase) and non-enzymatic (GSH) are presented in Table 1. A significant increase in the GSH and nonsignificant decrease in the SOD and catalase activities were observed in the CCl₄...
Joseph, et al.: Antioxidant activity of Phytocee™

**Table 1: Effect of Phytocee™ on SOD, catalase and GSH**

| Treatment groups                        | SOD (U/mg protein) | Catalase (U/mg protein) | GSH (μmoles/g liver) |
|----------------------------------------|--------------------|-------------------------|----------------------|
| Vehicle control (DM water; 10 ml/kg)   | 4.30±0.40          | 17.29±1.33              | 6.15±0.29            |
| CCl4 control (CCl4 and olive oil)      | 3.68±0.37          | 12.73±0.78              | 9.42±0.42*           |
| Vitamin C (20 mg/kg)                   | 5.00±0.39          | 17.24±1.43              | 7.83±0.57            |
| Phytocee™ (20 mg/kg)                   | 5.06±0.36          | 18.86±1.33              | 8.06±0.37            |
| Phytocee™ (100 mg/kg)                  | 4.59±0.53          | 17.76±2.54              | 8.05±0.24            |
| Phytocee™ (200 mg/kg)                  | 4.95±0.52          | 16.89±1.60              | 7.49±0.46*           |

Values are expressed as mean ± SEM, n = 6, *P ≤ 0.05 vehicle control versus CCl4 control, **P ≤ 0.05 CCl4 control versus treated groups, SEM = Standard error of the mean, SOD = Superoxide dismutase, GSH = Reduced glutathione, DM = Demineralized

**DISCUSSION**

Stress is an important concern in production animals, specifically in modern intensive farming systems. As a consequence, stress leads to deteriorated health, reduced performance, and productivity. Hence, it is suggested that antioxidant/pro-oxidant balance is responsible for maintaining animal health, productivity, and reproductive performance. The propensity for natural antioxidants is increasing as a result of the global trend of restricting the use of synthetic substances. In pursuit of the above, a novel polyherbal formulation Phytocee™ with ingredients well-known in ayurveda for antioxidant/antistress, adaptogenic, immunomodulatory activities was formulated. This study elucidated the antioxidant and protective activities of this polyherbal formulation. Carbon tetrachloride (CCl4) model was used to evaluate the antioxidant effects of Phytocee™ as it is one of the well-recognized and widely used animal models to investigate the antioxidant and protective effects.

CCl4 administration caused significant generation of free radicals/reactive intermediates evident from the significant increase in the MDA levels in CCl4 group when compared with vehicle control group. Of many biological targets of oxidative stress, lipids are the most involved class of biological molecules. The hepatic oxidative stress induced by CCl4 in our study is evident from the significant increase in the MDA levels as well as increase in the serum markers for hepatic injury ALT and AST. The significant decrease in the hepatic MDA levels, serum ALT and AST in the groups pretreated with Phytocee™ as compared with CCl4 treated group in the present study is indicative of the antioxidant potential of Phytocee™. The active ingredients present in the Phytocee™ were able to maintain homeostasis between free radicals produced and the antioxidants thereby protecting the liver from CCl4 damage.

The antioxidant defenses play a vital role in quenching reactive oxygen species. CCl4 administration reduced the SOD and catalase levels and Phytocee™ pretreated groups revealed marked increase in the SOD and catalase levels. Our findings are consistent with the previous reports.

Administration of CCl4 also increased the GSH levels significantly, which is consistent with the literature.
Groups pretreated with Phytocee™ modulated the GSH levels to within the normal limits[19] in rat liver.

Phytocee™ revealed its antioxidant effect by a significant reduction of MDA, ALT, and AST and by modulating the levels of enzymatic and nonenzymatic antioxidant defenses. Similar results obtained in studies on the individual ingredients of the Phytocee™ are previously reported.[34-36] Interestingly Phytocee™ could ameliorate LPO and increase the antioxidant enzymes comparable and much better than synthetic Vitamin C. Which may be attributed to the increased bioavailability of natural versus synthetic Vitamin C.[37,38]

In addition to the studies on the antioxidant activities of individual ingredients of Phytocee™, the polyherbal formulation is also efficacious against 2,2’-azobis [2-methylpropionamide] dihydrochloride induced oxidative stress using HepG2 cells in vivo and in vitro in cellular antioxidant assay.[13] Thus, our in vivo and in vitro study findings on Phytocee™ are correlated.

CONCLUSION

Our study supports the antioxidant potential of Phytocee™ and highlights its efficacy in the reversal of LPO, decreased levels of ALT, AST and modulation of enzymatic and nonenzymatic antioxidant defenses. The polyherbal formulation, Phytocee™ can be recommended as a natural antioxidant feed supplement to overcome stress-related effects.

REFERENCES

1. Rosales AG. Managing stress in broiler breeders: A review. J Appl Poult Res 1943;2:199-207.
2. Brunet-Rossinini AK. Reduced free-radical production and extreme longevity in the little brown bat (Myotis lucifugus) versus two non-flying mammals. Mech Ageing Dev 2004;125:11-20.
3. Sies H. Oxidative Stress: Oxidants and Antioxidants. New York: Academic Press; 1991. p. 2-8.
4. Botsoglou NA, Taitzoglou IA, Botsoglou E, Lavrentiadou SN, Kokoli AN, Roubies N. Effect of long-term dietary administration of oregano and rosemary on the antioxidant status of rat serum, liver, kidney and heart after carbon tetrachloride-induced oxidative stress. J Sci Food Agric 2009;89:1397-406.
5. Sahin K, Sahin N, Sari M, Gursu MF. Effects of vitamins E and A supplementation on lipid peroxidation and concentration of some mineral in broilers reared under heat stress (32°C). Nutr Res 2002;22:723-31.
6. Christaki E. Naturally derived antioxidants in poultry nutrition. Res J Biotechnol 2012;7:109-12.
7. Joshua AJ, Vijayabalaji V, Goudar KS, Sameera N, Amit A. Safety assessment of some herbal poultry formulations on acute exposure in rats. Indian J Nat Prod 2008;24:10-6.
8. Manju DK, Thangavel A, Leela V, Kalatharan J. Effect of dietary supplementation of amla and grape seed on semen characteristics of broiler breeder cocks. Tamil Nadu J Vet Anim Sci 2010;6:65-70.
9. Khan KH. Roles of Emblica officinalis in medicine — A review. Bot Res Int 2009;2:218-28.
10. Sethi J, Sood S, Seth S, Talwar A. Evaluation of hypoglycemic and antioxidant effect of Ocimum sanctum. Indian J Clin Biochem 2004;19:152-5.
11. Bhattacharya A, Ghosal S, Bhattacharya SK. Anti-oxidant effect of Withania somnifera glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J Ethnopharmacol 2001;74:1-6.
12. Chandrasekaran CV, Sundaranarjan K, David K, Agarwal A. In vitro efficacy and safety of poly-herbal formulations. Toxicol In Vitro 2010;24:885-97.
13. Tasaduq SA, Singh K, Sethi S, Sharma SC, Bedi KL, Singh J, et al. Hepatocurative and antioxidant profile of HP-1, a polyherbal phytopharmaceutical. Hum Exp Toxicol 2003;22:639-45.
14. Knight JA, Pieper RK, McClellan L. Specificity of the thiobarbituric acid reaction: Its use in studies of lipid peroxidation. Clin Chem 1988;34:2433-8.
15. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.
16. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogalol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
17. Aebi HE. Catalase in vitro. Methods Enzymol 1983;105:121-6.
18. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman’s reagent. Anal Biochem 1968;19:271-277.
19. Abeyesinghe SM, Wathes CM, Nicol CJ, Randall JM. The aversion of broiler chickens to concurrent vibrational and thermal stressors. Appl Anim Behav Sci 2001;73:199-215.
20. St-Pierre NR, Cobanov B, Schnitkey G. Economic losses from heat stress by US livestock industries. J Dairy Sci 2003;86:ES2-77.
21. Lykkesfeldt J, Svendsen O. Oxidants and antioxidants in disease: Oxidative stress in farm animals. Vet J 2007;173:302-11.
22. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr 2003;133:1286-90.
23. Huang Q, Zhang S, Zheng L, He M, Huang R, Lin X. Hepatoprotective effects of total saponins isolated from Taraxochlamys affinis against carbon tetrachloride induced liver injury in rats. Food Chem Toxicol 2012;50:713-8.
24. Hsu LS, Ho HH, Lin MC, Chyau CC, Peng JS, Wang CJ. Mulberry water extracts (MWEs) ameliorated carbon tetrachloride-induced liver damages in rats. Food Chem Toxicol 2012;50:3086-93.
25. Fouw JD. Environmental Health Criteria 208, Carbon Tetrachloride. Geneva: World Health Organization; 1999.
26. Georgieva NV. Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems — A review. Bul J Vet Med 2005;8:1-11.
27. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 2005;15:316-28.
28. Ashiya GS, Kotagale NR, Wadodkar SG, Dorle AK. Hepatoprotective activity of Panchagavya ghrita against carbon tetrachloride induced hepatotoxicity in rats. Indian J Pharmacol 2003;35:308-11.
29. Surendran S, Eswaran MB, Vijayakumar M, Rao CV. In vitro and in vivo hepatoprotective activity of Cissampelos pareira against carbon tetrachloride induced hepatic damage. Indian J Exp Biol 2011;49:938-45.
30. Eidi A, Mortazavi P, Bazargan M, Zaringhalam J. Hepatoprotective activity of Cinnamon ethanolic extract against CCl4-induced liver injury in rats. EXCLI J 2012;11:495-507.
31. Nakagawa K. Carbon tetrachloride-induced alterations in hepatic glutathione and ascorbic acid contents in mice fed a diet containing ascorbate esters. Arch Toxicol 1993;67:886-90.
32. Lai TY, Weng YJ, Kuo WW, Chen LM, Chung YT, Lin YM, et al. Taoho Chengqi Tang ameliorates acute liver injury induced by carbon tetrachloride in rats. Zhong Xi Yi Jie He Xue Bao 2010;8:49-55.

Journal of Natural Science, Biology and Medicine | January 2015 | Vol 6 | Issue 1
33. Lu SC. Regulation of hepatic glutathione synthesis: Current concepts and controversies. FASEB J 1999;13:1169-83.
34. Panda S, Kar A. Fruit extract of *Emblica officinalis* ameliorates hyperthyroidism and hepatic lipid peroxidation in mice. Pharmazie 2003;58:753-5.
35. Ramesh B, Satakopan VN. Antioxidant activities of hydroalcoholic extract of *Ocimum sanctum* against cadmium induced toxicity in rats. Indian J Clin Biochem 2010;25:307-10.
36. Harikrishnan B, Subramanian P, Subash S. Effect of *Withania somnifera* root powder on the levels of circulatory lipid peroxidation and liver marker enzymes in chronic hyperammonemia. E J Chem 2008;5:872-7.
37. Raghu V, Platel K, Srinivasan K. Comparison of ascorbic acid content of *Emblica officinalis* fruits determined by different analytical methods. J Food Compost Anal 2007;20:529-33.
38. Vinson JA, Bose P. Comparative bioavailability of synthetic and natural Vitamin C in guinea pigs. Nutr Rep Int 1983;27:875-80.

*How to cite this article:* Joseph JA, Radakrishnan U, Mutyala S, Goudar KS, Thachappully Ayyappan UP, Agarwal A. Antioxidant and protective effects of Phytocee™ against carbon tetrachloride-induced oxidative stress. J Nat Sc Biol Med 2015;6:183-7.

*Source of Support:* Nil. *Conflict of Interest:* None declared.