Research Article

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

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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 (CCl_4) at 2 ml/kg as a single dose orally to all groups except the vehicle control group. After 24 h of administration of CCl_4, 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 CCl_4 treated group. **Conclusion:** The findings suggest that Phytocee™ markedly reversed the effects of CCl_4 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

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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 (CCl_4) (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 tartarate, 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 (CCl_4 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 CCl_4 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[14] 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 $P \leq 0.05$.

RESULTS

The mean hepatic malondialdehyde (MDA) levels are shown in Figure 1. The CCl_4 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 ($P \leq 0.05$) decrease in the hepatic MDA levels as compared with the CCl_4 control group.

Mean serum ALT and AST levels, which are markers for hepatic tissue damage are represented in Figure 2. The CCl_4 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 CCl_4 group ($P \leq 0.05$).

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 CCl_4

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
CCl_4 control (CCl_4 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±1.93	8.06±0.37
Hytocee™ (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, $n = 6$, *$P \leq 0.05$ vehicle control versus CCl_4 control, #$P \leq 0.05$ CCl_4 control versus treated groups, SEM = Standard error of the mean, SOD = Superoxide dismutase, GSH = Reduced glutathione, DM = Demineralized

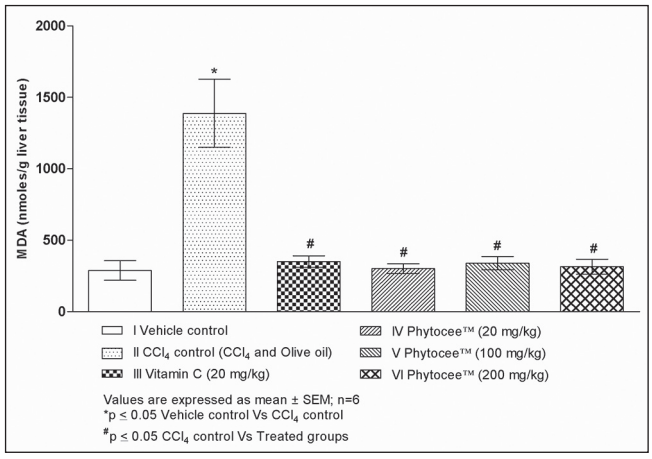

Figure 1: Effect of Phytocee™ on hepatic malondialdehyde

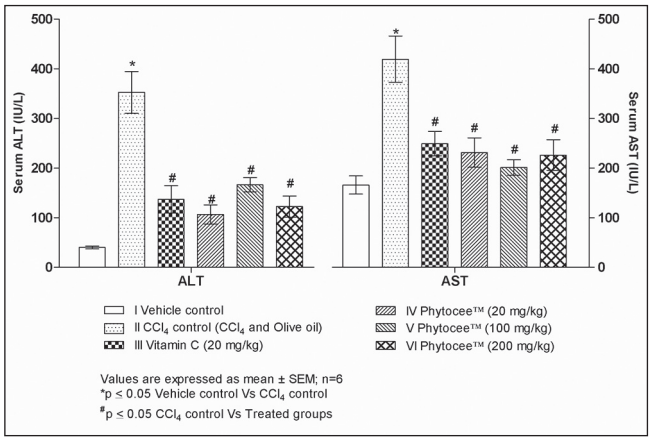

Figure 2: Effect of Phytocee™ on serum alanine aminotransferase and aspartate aminotransferase

control group when compared to vehicle control group. Phytocee™ pretreated groups modulated the GSH levels with Phytocee™ at the dose of 200 mg/kg revealing significant antioxidant effect. Phytocee™ pretreatment increased the activities of enzymatic antioxidant defenses i.e., SOD and catalase.

DISCUSSION

Stress is an important concern in production animals, specifically in modern intensive farming systems.[19,20] As a consequence, stress leads to deteriorated health, reduced performance, and productivity.[21] 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.[22] 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 (CCl_4) 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.[23-25]

CCl_4 administration caused significant generation of free radicals/reactive intermediates evident from the significant increase in the MDA levels in CCl_4 group when compared with vehicle control group. Of many biological targets of oxidative stress, lipids are the most involved class of biological molecules.[26,27] The hepatic oxidative stress induced by CCl_4 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.[28] The significant decrease in the hepatic MDA levels, serum ALT and AST in the groups pretreated with Phytocee™ as compared with CCl_4 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 CCl_4 damage.

The antioxidant defenses play a vital role in quenching reactive oxygen species. CCl_4 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.[29,30]

Administration of CCl_4 also increased the GSH levels significantly, which is consistent with the literature.[31,32]

Groups pretreated with Phytocee™ modulated the GSH levels to within the normal limits[33] 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-methyl propionamidine] dihydrochloride induced oxidative stress using HepG2 cells *in vitro* in cellular antioxidant assay.[12] 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.

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