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Original Research Article

Comparative Efficacy of Phytotherapeutic, Probiotic, and Micronutrient Strategies in the Mitigation of DEHP-Induced Systemic Toxicity

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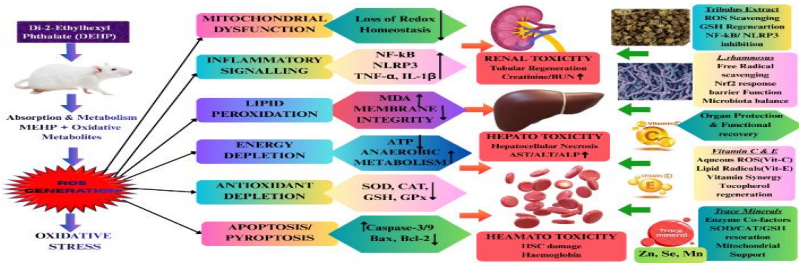
Abstract: Di(2-ethylhexyl) phthalate (DEHP), an ubiquitous environmental toxin and endocrine disruptor is commonly used as a plasticizer in polyvinyl chloride (PVC) products. Exposure to DEHP has been shown to cause significant oxidative stress and tissue damage in the hematopoietic, hepatic, and renal systems. This study evaluated the efficacy of three different interventions (Lactobacillus rhamnosus (1×10^8 CFU/kg bw), a hydro-methanolic (70:30) extract of Tribulus terrestris fruit (200 mg/kg bw), and a combination of antioxidant micronutrients; i.e., vitamin E (100 mg/kg bw), vitamin C (100 mg/kg bw), zinc (20 mg/kg bw), selenium (1 mg/kg bw), and manganese (10 mg/kg bw)) on the toxicity induced by DEHP in male albino Wistar rats. Nine experimental groups were studied; these included: a control group, an experimental group treated with DEHP, and six experimental groups treated with single interventions. Haematological parameters (hemoglobin, RBCs, hematocrit, WBCs, differential counts), tissue hepatic enzymes (AST, ALT, ALP, ACP), serum concentrations of renal function markers (serum creatinine, BUN), and tissue antioxidant enzyme activity (SOD, CAT, GST, GPx, GR, G6PDH, SDH, PDH, LDH) were measured after 20 days of experimentation. Results demonstrated that acute DEHP exposure for 20 days resulted in a significant decrease in haematological parameters (39% reduction in haemoglobin levels), increased liver enzymes (47-59% increases in ALT/ALP), decreased renal function, and reduced antioxidant defence mechanisms in the liver and kidneys. The Tribulus terrestris extract showed greater therapeutic efficacy than either L. rhamnosus or the antioxidant micronutrients, restoring 71.4% of the lost hemoglobin levels, and 71.6% of the lost hepatic glutathione. Although both L. rhamnosus and the antioxidant micronutrients demonstrated moderate degrees of protection, with 65-70% recovery indices, individual micronutrient studies also demonstrated a dose-response relationship, and the selenium and zinc combinations exhibited synergism in their antioxidant capabilities. Collectively, these results suggest that a polyherbal-probiotic combination may represent a useful rapid acting therapy for minimizing DEHP-induced multi-organ toxicities via enhanced antioxidant defence mechanisms and restoration of cellular redox homeostasis during a limited 20-day intervention.

Keywords: DEHP, oxidative stress, Tribulus terrestris, Lactobacillus M rhamnosus, antioxidants, hepatotoxicity, nephrotoxicity, hematopoiesis, acute toxicity.

Graphical Abstract



INTRODUCTION

Plasticisers, such as Di(2-ethylhexyl) phthalate (DEHP), are found in many polyvinyl chloride (PVC) products (An et al., 2024; Zöngür, 2024) and can migrate into the environment and then into the human body through either oral ingestion or inhalation, and dermal contact (Ding, S., Qi, et al., 2021; Rowdhwal & Chen, 2018). After being absorbed, DEHP is quickly broken down in the liver and gut into Mono-Ethylhexylphthalate (MEHP) and other oxidative metabolites (Chen et al., 2022; Koch et al., 2005). The parent compound has a short half-life, but repeated exposure keeps the active metabolites in the body, which can cause cumulative toxicity in different organ systems (Rousseau-Ralliard et al., 2024; Aydemir et al., 2024; Bijjala et al., 2025). Chronic human exposure is significantly correlated with metabolic disorders, such as diabetes, hyperlipidemia, insulin resistance, and non-alcoholic fatty liver disease (NAFLD) (Robles-Matos et al., 2023; He et al., 2023; Carli et al., 2022).

The liver is the main site of DEHP metabolism and a major target for its toxicity (Singh, Pandey, et al., 2025). Mechanisms behind the liver damage induced by DEHP include an interaction among oxidative stress, mitochondrial dysfunction and inflammation (Behairy et al., 2021; Fang et al., 2025; Guo, Deng, et al., 2024; Zhang, Zhao, et al., 2022). More specifically, DEHP induced liver failure occurs through the activation of thioredoxin-interacting protein (TXNIP), which inhibits important transcriptional factors (PGC-1α, TFAM and NRF1) in mitochondrial biogenesis while activating the NLRP3 inflammasome (Liu, Han, et al., 2021; Xu et al., 2024). TXNIP-mediated signalling activates the NF-κB/MAPK pathway and generates ROS, inducing apoptosis and dysfunction in hepatocytes (Dagdeviren et al., 2023; Xu et al., 2024).

The manifestation of liver damage is evidently dose-dependent. Steatosis (lipid deposition) typically arises with moderate DEHP dosing, whereas high exposure results in significant cellular damage and necrosis, with minimal lipid deposition (Robles-Matos et al., 2023), indicating a metabolic transition from lipid storage to oxidative stress (ROS)-induced injury (Wang et al., 2025; Yang et al., 2025). Furthermore, DEHP can provoke an inflammatory response in Kupffer cells, leading to elevated levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) that may contribute to cholestasis and bile duct damage via metabolic reprogramming (Yu et al., 2025; Wu, Zhang, et al., 2022).

DEHP causes kidney injuries through several mechanisms. DEHP triggers oxidative and inflammatory pathways to cause damage to the kidneys (Ding, Huang, et al., 2023), as well as cellular pathways that include stress kinase pathways and transcription factor pathways (Kandel et al., 2025). Exposure to DEHP leads to the activation of p38 MAPK and NF-κB signalling pathways in the kidney, causing the inflammation and destruction of podocytes (Shi et al., 2023). Another mechanism of DEHP injury to the kidneys is through the activation of the NLRP3 inflammasome and caspase-1, which drive a type of programmed cell death known as pyroptosis in renal tubular cells that is characterized by the cleavage of gasdermin-D (Li, Gu, et al., 2024; Wei et al., 2025).

The increase in the production of IL-1β, TNF-α, and caspase-1 in the kidneys due to exposure to DEHP are the indicators of damage caused by DEHP is due to the activation of the inflammasome (Aranda-Rivera et al., 2022; Wei et al., 2025). DEHP also causes intrinsic apoptosis in renal epithelial cells through an increase in the expression of pro-apoptotic proteins (Bax, caspase-3), whereas it has a decrease in the expression of anti-apoptotic protein (Bcl-2) (Maire et al., 2005; Ran et al., 2025). The histological manifestations of this molecular injury include tubular atrophy, glomerular hypertrophy, interstitial fibrosis, and leukocyte infiltration (Liu, Zhang, et al., 2025). Thus, DEHP's nephrotoxicity is due to the loss of the balance between ROS/Nrf2, activation of MAPK and NF-κB signalling pathways, NLRP3/caspase-1 induced pyroptosis and intrinsic apoptosis (Bax/Bcl-2/caspase-9/3) (Gad El-Karim et al., 2023; Singh, Jangra, & Kumar, 2024; Pan et al., 2025).

DEHP influences blood cell production by inducing an inflammatory environment in the bone marrow and disrupting redox balance thereby interfering with signalling pathways (Tsai et al., 2022; Hu et al., 2023). Inflammation and oxidative stress arise when DEHP-induced reactive oxygen species (ROS) stimulate the NF-

κB pathway and disturb the normal cytokine equilibrium in the bone marrow (Kaiser et al., 2021; Lingappan, 2018). Oxidative stress and the consequent disturbance of cytokine equilibrium are associated with heightened apoptosis in bone marrow cells, reductions in specific leukocyte and lymphocyte populations, and elevations in neutrophils, ultimately resulting in alterations in peripheral blood counts (Chakraborty et al., 2023; Chi et al., 2024; Manz et al., 2015).

High dose DEHP is known to affect vascular endothelial growth factor (VEGF) signalling, and to induce an attenuation in the expression of genes related to erythropoiesis/myelopoiesis thereby reducing the number of hematopoietic stem cells (HSCs), and also reducing the circulating population of erythrocytes (Hu et al., 2023). Therefore the reduction in angiogenic support, and the induction of apoptosis in progenitor cells clearly represent a mechanistic explanation for DEHP-induced haematotoxicity (Hussein et al., 2023). Importantly, these mechanisms were noted at environmentally relevant doses and therefore it is imperative that there be a rapid and extensive evaluation of possible protective interventions (Soni et al., 2022).

Given that the cause of toxicity from exposure to DEHP would be due to oxidative stress, mitochondrial dysfunction, inflammation, pyroptosis/apoptosis, and a disruption in metabolism, there is rationale for using antioxidants, anti-inflammatory drugs, and mitochondrial protective compounds to either prevent or diminish the negative effects caused by exposure to DEHP.

Lactobacillus rhamnosus is an effective probiotic for its antioxidant and immunomodulatory effects (Steele, 2022; Rocha-Ramírez et al., 2021). In addition, oral administration of *L. rhamnosus* was demonstrated to be capable of enhancing antioxidant defenses in both the liver (Chen, Kong et al., 2022) and bone marrow (i.e., through increasing levels of MnSOD and AMPKα) as well as supporting mitochondrial function (López-Almada et al., 2024; Zhang, Sun, et al., 2024; Zhang, Wang, et al., 2015). *L. rhamnosus GG* restored redox balance and elevated Higd2a mRNA expression and MnSOD1 protein levels in both the liver and bone marrow of ethanol exposed rats (Salazar et al., 2025). Moreover, *L. rhamnosus GG* increases gut microbiota diversity and decreases endotoxemia indicating that it could possibly protect against systemic DEHP toxicity by way of the gut-liver axis and Nrf2 mediated signalling (Sun et al., 2025; Baralić et al., 2020; Zheng et al., 2022; Guo, Yu, et al., 2023).
Extracts of *Tribulus terrestris* fruit are a rich source of steroidal saponins and flavonoids(Saeed et al., 2024). There are many studies that demonstrate *Tribulus terrestris* has significant hepatoprotective as well as nephroprotective properties (Mohy-Ud-Din & Jonassaint, 2024). *Tribulus terrestris* normalizes serum liver enzymes (AST and ALT), and serum creatine levels in toxicological studies without any adverse effects being observed in tissue histopathology (Darbandi & Jabbar, 2025; Al-Mohamadi et al., 2025). The protective actions of *Tribulus terrestris* include restoring glutathione (GSH) levels, inhibiting lipid peroxidation, and inhibiting the NF-κB/IL-6 inflammatory signalling pathways (Shetty et al., 2024; Abdi et al., 2025; Chen, Deng, et al., 2025).

Vitamin C is a powerful antioxidant that can neutralize free radicals in the aqueous phase of the body and also acts to regenerate other antioxidants (e.g. Vitamin E) (Alberts et al., 2025). It has nearly completely restored DEHP-disrupted metabolic pathways, blood glucose and lipid profiles and depleted antioxidant enzyme activity, for instance, superoxide dismutase (SOD) (Manokaran et al., 2025; Soni et al., 2022; Mo et al., 2024). The reduction of reactive oxygen species (ROS) in plasma by Vitamin C, and its inhibition of oxidative DNA damage, and subsequent inflammatory cascades make Vitamin C a crucial first line of defense (Kawashima et al., 2015; Gęgotek & Skrzydlewska, 2022; Ibrahim et al., 2022).
Vitamin E and Selenium are synergistic when used together. Vitamin E protects the cell membranes from peroxidation and Selenium is essential for the glutathione peroxidase (GPx) that breaks down hydro peroxides (Kilicarslan You et al., 2024; Shahidin et al., 2025). Co-treatment with both Vitamins E and Selenium has been shown to greatly reduce DEHP-induced toxicity and normalize blood glucose levels and liver function tests, and decrease spleen damage more effectively than either treatment separately (Haque et al., 2025). In summary, they provide a comprehensive defence mechanism against oxidative injury (Ding, Sun, et al., 2025; Xiao et al., 2021) and can limit phthalate-induced harm" (Alam & Hoque, 2018).

Zinc and manganese are two essential trace minerals that serve as cofactors for several key antioxidant enzymes (El-Sayed et al., 2024). Zinc is required for Cu/Zn superoxide dismutase (SOD) activity and increase expression of metallothionein, which will bind free radicals (Hübner & Haase, 2021; Briassoulis et al., 2023). Manganese is necessary to have active mitochondrial Mn-SOD(SOD2) activity (Grujicic & Allen, 2025). The SOD/GPx enzyme system is replaced by Zn, Se, and Mn in detoxification (Manful et al., 2025). Therefore, supplementing Zn and Mn can also restore endogenous enzymatic defenses and reduce the amount of ROS produced at the mitochondria due to DEHP exposure (Oyovwi et al., 2025; Xia et al., 2024).

The hypothesis of this study is that the multiorgan oxidative stress caused by DEHP are due to overlap in pathways of oxidative stress, inflammation and metabolic alterations and its exposure can be significantly reduced using pharmacologic and nutritional interventions that target both enzymatic and non-enzymatic antioxidant defence mechanisms, inflammatory signalling pathways, mitochondrial energy production, and changes in the gut microflora to restore the mucosal barrier function. This comprehensive study compares the effectiveness of seven different protective treatments (*L.rhamnosus, T. terrestris,* Vitamins C and E, Se, Zn, Mn) when administered singly to compare their relative effectiveness and to determine those treatments that should be examined further as part of multi-modal treatment regimens including synergetic combinations of botanicals, probiotics and micronutrient based treatments.

MATERIALS AND METHODS

Chemicals

Di(2-ethylhexyl) phthalate (DEHP), 99% purity, was purchased from Sigma-Aldrich (India). All other chemicals like Vitamin E (α-tocopherol acetate), vitamin C (L-ascorbic acid), Zinc as Zinc chloride($ZnCl_2$), Selenium as Sodium Selenite (Na_2SeO_3), and Manganese as Manganese Sulfate Monohydrate and solvents utilized were analytical grade and also purchased from Sigma-Aldrich, India.

Preparation of Hydro-methanolic fruit extract of *Tribulus terrestris*

Fruits of *Tribulus terrestris* (TT) were collected from pesticide-free local fields and taxonomically identified by the staff from Department of Botany, SVU College of Sciences, Sri Venkateswara University, Tirupati; the ds were also authenticated by the same department and a voucher specimen (Code No. SVUH:0046) was kept in the herbarium for future reference. The fruits were carefully cleaned, then air-dried in the shade at room temperature to avoid damage to thermally labile phytochemicals such as some saponins or flavonoids. The air-dried materials were then pulverized utilizing a commercial grinder and ground into a fine powder that was then passed through a sieve to ensure consistent particle size to optimize the extraction process. The final powdered material was stored in sealed airtight containers at room temperature until the extraction was carried out.

The fruit powder 100 g was immersed in 1000 mL hydro-alcoholic solution with 70% methanol and 30% distilled water in a conical flask stoppered with cotton plug. The conical flask was then kept in the orbital shaker for 72 hrs. at 150 rpm. Then extract was first filtered through muslin cloth and then through Whatmann filter paper no.1. The filtrate so obtained was collected in the large petri dish allowed to evaporate to obtain semisolid mass of extract. The extract so obtain was kept in desiccator for further use. The required concentration of extract used in study was prepared in normal saline.

Preparation of *Lactobacillus rhamnosus*:

Standard Probiotic Culture *L. rhamnosus* GG (MTCC 53103) was obtained from MTCC (Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India), MTCC Code 53103 in powder form in sealed glass vials and stored in Refrigerator at 4°C. and prepared as an aqueous suspension in sterile phosphate-buffered saline (PBS, pH 7.2) containing 1×10^8 colony-forming units (CFU) per kilogram body weight. The probiotic suspension was administered orally via gavage daily.

Animals Maintenance

Preparation of tissue homogenates

The liver and kidney tissue from each rat were placed in 10 ml/g ice-cold phosphate-buffer saline (pH 7.5) and homogenized using a glass homogenizer. The homogenates were then centrifuged at 10,000 g for 10 min at 4°C, and the resultant supernatants were collected for later detection of biochemical parameters by following the under mentioned standard methodologies.

S.NO	PARAMETER	METHOD
1.	Superoxide Dismutase (SOD)	Misra and Fridovich, (1972)
2.	Catalase (CAT)	Aebi (1984)
3.	Glutamate Pyruvate Transaminase (GPT)	Reitman and Frankel (1957)
4.	Glutathione-S-Transferase (GST)	Habig et al. (1974).

Adult male rats (weighing 225±10 g) were selected in the present study and were housed in stainless steel mesh cages in a contained laboratory environment (i.e. temperature 23±2°C, relative humidity 50±5%, 12:12 light/dark cycle). Standard rat chow and tap water were provided ad libitum (Sai Feeds, Bangalore, India). Rats were acclimated to the laboratory conditions for a period of one week. Approval for the experimental protocols was granted by the Institutional Animal Ethics Committee (Resolution No. DRBILS/IAEC/LS/2025/08 dated 25.01.2025), Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Experimental Design and Treatment Protocol

A total of 54 Male albino Wistar rats (n=6) were randomly divided into eight groups (n = 6 per group)::

T1- Control: Received vehicle only (corn oil, 2 ml/kg body weight via oral gavage, daily)

T2- DEHP: Received DEHP 500 mg/kg body weight in corn oil via oral gavage, daily

T3- DEHP 500 mg/kg + *Tribulus terrestris* fruit extract 200 mg/kg body weight

T4- DEHP 500 mg/kg + *L. rhamnosus* 1×10^8 CFU/kg body weight

T5- DEHP 500 mg/kg + Sodium selenite 1 mg/kg body weight

T6- DEHP 500 mg/kg + Zinc sulfate 20 mg/kg body weight

T7- DEHP 500 mg/kg + Manganese sulfate 10 mg/kg body weight

T8- DEHP 500 mg/kg + Vitamin E 100 mg/kg body weight

T9- DEHP 500 mg/kg + Vitamin C 100 mg/kg body weight

After 20 days of treatment, the overnight fasted animals were sacrificed under light ether anesthesia. Their body weights were recorded. Blood was collected by cardiac puncture. Serum was separated and stored at -20°C for biochemical estimations. Liver was excised immediately, cleaned in ice-cold normal saline, blotted and weighed on digital balance.

Body and Organ weight measurements

The body weight has been recorded on the initial day of experiment and also on the day of sacrifice (20^{th} day), both the Control and Experimental groups, by using automatic Electronic Balance. Blood was collected in sterile tubes by cardiac puncture for hormone and hematological studies. Similarly the weight of organ (Liver and Kidney) was also recorded.

Hepatosomatic index

The relative weight of the organ (Liver & Kidney) was calculated using the formula,

$$\text{Hepatosomatic index} = \frac{\text{Weight of organ}}{\text{Total Body weight}} \times 100$$

5.	Glutathione peroxidase (GPx)	Flohe and Gunzler (1984)
6.	ReducedGlutathione (GSH)	Theodorus et al., (1981)
7.	Glutathione Reductase (GR)	Ellman (1959)
8.	Lactate Dehydrogenase (LDH)	Nachlas et al., (1960)
9.	Glucose-6-Phosphate Dehydrogenase (G-6-PDH)	George and Waller (1965).
10.	Succinate Dehydrogenase (SDH)	Nachlas et al. (1960).
11.	Pyruvate Dehydrogenase (PDH)	Chretien et al., (1995)
12.	Aspartate Amino Transferase (AST)	Reitman and Frankel (1957)
13.	Alanine Amino Transferase (ALT)	Reitman and Frankel (1957) as described by Berg Meyer and Bernt (1965).
14.	Alkaline Phosphatase (ALP):	Bodansky (1932)
22.	Acid Phosphatase (ACP)	Hillman (1971)

Haematological Analysis

For the haematological examination, Blood samples were collected from the retro orbital plexus of the rats using an heparinized capillary tube. The haematological analyses were measured with an auto cell counter for veterinary purposes, specific for rat (MS9-5 of Melet Schloesing Lab, New Delhi, India).

Serum Biochemical Parameters

For biochemical investigations serum was separated by centrifugation at 4000 rpm for 10 minutes and kept at -4 °C. Serum biochemical analyses were conducted using standard colorimetric and enzymatic methods on an automated biochemistry analyzer (Hitachi 912, Roche, Switzerland):

Renal Function Markers: Serum creatinine (mg/dl): serum creatinine was estimated by alkaline Jaffe's Picrate method and Blood urea nitrogen (BUN, mg/dl) estimated by enzymatic urease method

All analyses were performed using commercially available diagnostic kits according to manufacturer's instructions with appropriate quality controls. Absorbance measurements were obtained at appropriate wavelengths using a spectrophotometer (Hitachi U-2800) with temperature control at 37°C.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) for six animals per group. Statistical significance between groups was evaluated using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). Differences were considered significant at $p<0.05$, very significant at $p<0.01$, and highly significant at $p<0.001$. Percent deviation over control (PDC) and percent deviation over DEHP-treated values (PDE) were calculated for comparative interpretation.

Percent Deviation from Control (PDC) was calculated for each parameter as:

PDC (%) = [(Treated group mean - Control group mean) / Control group mean] × 100

Percent Deviation from DEHP (PDE) was calculated for each treated group as:

PDE (%) = [(Treated group mean - DEHP group mean) / DEHP group mean] × 100

Percent Recovery (PR) was calculated for each parameter using the formula:

PR (%) = [(Treated group mean - DEHP group mean) / (Control group mean - DEHP group mean)] × 100

RESULTS

Growth Performance and Metabolic Parameters

Compared to the Control Group (T1), DEHP treated rats (T2) had statistically significant depressed performance in body weight gain, which averaged 30.35 % less than the Control group (Control = 254.34 ± 7.06 g; DEHP = 177.15 ± 4.91 g, $p < 0.001$). Rats exposed to DEHP experienced severe suppression in weight gain compared to Controls (Loss in DEHP = 47.19 ± 1.31 g; Gain in Controls = 31.08 ± 0.86 g); this represented a 51.83 % deviation below that of the Controls. As shown in Table 1, food intake in DEHP-treated rats decreased by 25.83 % to 15.22 ± 0.42 g / 100 g bw / day; water consumption decreased by 28.76 % to 22.17 ± 0.61 mL / rat / day; and urine production decreased by 32.20 % to 4.19 ± 0.12 mL / rat / day, all of which were significantly ($p < 0.001$) reduced relative to the Controls.

As illustrated in Table 1, *Tribulus terrestris* (T3) provided a superior degree of restoration of growth related parameters to the baseline level (70.88 % restoration of final body weight = 231.86 ± 6.42 g; 70.45 % restoration of weight gain / loss; 73.96 % restoration of food intake; and 70.95 % restoration of water consumption). The restoration of growth related parameters was slightly less than T3 in the *Lactobacillus rhamnosus* (T4) group (62.29 % restoration of final body weight; 66.29 % restoration of composite metabolic parameters). Vitamin E (T8) restored 53.48 % of final body weight. The supplemental trace minerals (T5-T7) exhibited progressive decreases in restoration capability, with the manganese supplement (T7) exhibiting only 30.52 % restoration of final body weight. In addition to final body weight, the restoration of water and urine parameters was consistent with the pattern observed with growth related parameters.

Antioxidative Defense Mechanisms In The Liver

The liver's defense mechanisms against oxidative stress were severely affected by DEHP exposure; SOD activity was significantly reduced (by 45%) to 34.19 ± 0.95 U/mg protein ($p < 0.001$); catalase activity was reduced by 47% to 46.61 ± 1.30 µ moles of H_2O_2 degraded/mg protein/min; GPx activity was increased (by 43%) to 26.20 ± 0.73 µ moles of NADPH oxidized / mg protein / min. Glutathione-S-transferase activity was also increased (by 46%) to 7.90 ± 0.22 µ moles of thioether formed/ mg of protein/min. However, reduced glutathione (GSH) levels were significantly reduced (to 53%) to 7.670 ± 0.213 µ moles/gm wet weight of the tissue; glutathione reductase activity was significantly reduced (by 43%) to 3.560 ±

0.099 units/mg protein ($p<0.001$).

Treatment with *Tribulus terrestris* resulted in the most complete recovery of the liver's antioxidative balance as evidenced by an increase of 69.9% in SOD activity, 73.1% in CAT activity, 74.8% in GPX activity, and 71.39% in GSH levels. The recovery values for SOD activity for *L. rhamnosus* (66.6%), CAT activity for *L. rhamnosus* (67.5%), GPx activity for *L. rhamnosus* (68.7%) and GSH levels for *L. rhamnosus* (68.4%) were very similar to those for Tribulus terrestris, although somewhat lower in each case. The recovery values for SOD activity for vitamin E (54.2%) and vitamin C (40.1%) were both less than that of *Tribulus terrestris* and *L. rhamnosus*. Individually administered trace elements had varying effects on the recovery of liver antioxidative balance with selenium resulting in a 38.5% recovery of SOD activity, zinc a 30.8% recovery of SOD activity, and manganese only a 25.2% recovery of SOD activity.

Mitochondrial Function And Energy Metabolic Enzymes of the Liver

Hepatic mitochondria are involved in the synthesis of ATP and other high-energy compounds through the process of cellular respiration. DEHP exposure was found to have profoundly impacted the ability of these organelles to perform their functions. Specifically, glucose-6-phosphate dehydrogenase (G6PDH) activity was increased (by 63.4%) to 12.79 ± 0.36 µ moles of formazan formed/mg protein/min ($p<0.001$), indicating a compensatory mechanism to help restore cellular energy status. Succinate dehydrogenase (SDH) activity was decreased (by 52%) to 1.41 ± 0.04 ($p < 0.001$); pyruvate dehydrogenase (PDH) activity was decreased (by 45%) to 1.29 ± 0.04; lactate dehydrogenase (LDH) activity was increased (by 41.9%) to 1.72 ± 0.05 ($p<0.001$). This suggests that oxidative phosphorylation is being disrupted and energy is being produced through anaerobic glycolysis rather than aerobic oxidation of carbohydrates.

Tribulus terrestris demonstrated the most significant effect on restoring mitochondrial and energy metabolic enzymatic function of all treatments tested as indicated by its comparative efficiency index (CEI = 0.7021) which was the highest CEI observed among the treatments tested. *Tribulus terrestris* demonstrated 71.96% recovery of G6PDH activity, 71.61% recovery of SDH activity, 71.85% recovery of PDH activity, and 69.86% recovery of LDH activity. *L. rhamnosus* demonstrated recoveries of 65.31% for G6PDH activity and 64.17% for SDH activity. Vitamin E demonstrated a moderate level of efficacy (CEI = 0.5457) in terms of restoring mitochondrial and energy metabolic enzymatic function of the liver. Manganese demonstrated the least effective treatment (CEI = 0.2998) in this regard.

Hepatic Marker Enzymes and Liver Function

AST was increased to 122.67±3.41 µmoles pyruvate/g wet tissue/min (a 47.00% increase above control; 83.45±2.32; $p<0.001$). The level of ALT was increased to 58.18±1.62, suggesting severe liver damage through cytoplasmic enzyme release by the damaged hepatocytes. The elevated levels of both enzymes in addition to the increases in ALP (to 7.52±0.21 µmoles of p-nitrophenyl formed / gram wet weight of tissue / min, a 46.02% elevation) and ACP (to 16.15±0.45, a 44.97% elevation over the control value of 11.14±0.31) provide evidence for significant disruption of liver function and morphology (Table 6).

The *Tribulus terrestris* treated group had a 96.11±2.67 AST recovery (a 67.72% recovery); 45.69±1.27 ALT recovery (a 69.18% recovery); 12.60±0.35 ACP recovery (a 70.86% recovery); and 5.92±0.16 ALP recovery (a 67.51% recovery). In comparison, *Lactobacillus rhamnosus* had a 60.17% AST recovery and a 65.72% ALT recovery. Vitamin E also demonstrated some degree of recovery, but this was less than that observed for *Tribulus terrestris* (57.09% AST recovery), and individual trace elements also demonstrated varying degrees of efficacy. Zinc provided the least amount of recovery (35.77% AST recovery), whereas selenium provided a 45.08% AST recovery and manganese only a 28.61% AST recovery.

Renal Antioxidant Enzyme Status

Antioxidant enzymes within the kidney exhibited suppressed activities similar to those seen in the liver. Renal superoxide dismutase (SOD) activity was decreased to 25.72±0.72 units/mg protein (a 43.02% decrease from the control value of 45.14±1.26, $p<0.001$). Catalase activity was decreased to 35.85±1.00 (a 45.00% decrease from the control value of 65.19±1.81), and glutathione-S-transferase (GST) activity was decreased to 2.09±0.06 (a 44.11% decrease from the control value of 3.74±0.10). Glutathione peroxidase (GPx) activity, however, was increased to 18.04±0.50 (a 45.01% increase over the control value of 12.44±0.35). Glutathione (GSH) was reduced to 3.948±0.110 Reduced Glutathione (GSH)- µ moles/gm wet weight of the tissue (a 47.01% decrease from the control value of 7.450±0.207), and glutathione reductase activity was increased to 18.038±0.502 units/mg protein (a 45.00% increase over the control value of 12.445±0.347), indicating that there is an activation of the cellular response to oxidative stress, which does not appear sufficient to mitigate the toxic effects associated with DEHP exposure (Table 4).

The *Tribulus terrestris* treated group demonstrated complete recovery of renal antioxidant enzyme activity as evidenced by SOD activity being restored to 39.46±1.10 (a 70.75% restoration), catalase activity being restored to 56.02±1.56 (a 68.73% restoration), GST activity being restored to 3.25±0.09 (a 72.50% restoration), and GSH being restored to 6.443±0.179 (a 71.248% restoration). Similarly, *Lactobacillus rhamnosus* demonstrated recovery of 60.76% for SOD and 66.89% for catalase. The mean percent recovery (MPR) data indicate that *Tribulus terrestris* demonstrated an overall recovery of 70.21% for renal antioxidant enzyme activity when averaged across the four measured enzyme activities, compared to 62.77% for *Lactobacillus rhamnosus* and 54.57% for vitamin E.

Renal Mitochondrial and Energy Metabolism Enzymes

Mitochondrial enzyme activities within the kidney were similarly disrupted. G6PDH activity was increased to 6.760±0.188 µ moles of formazan formed/mg protein/min (a 42.92% increase over the control value of 4.730±0.132), succinate dehydrogenase (SDH) activity was decreased to 0.983±0.027 (a 47.01% decrease from the control value of

1.855±0.052), pyruvate dehydrogenase (PDH) activity was decreased to 1.010±0.028 (a 42.05% decrease from the control value of 1.743±0.048), lactate dehydrogenase (LDH) activity was increased to 1.254±0.035 (a 39.96% increase over the control value of 0.896±0.025) (Table 5). The *Tribulus terrestris* treated group demonstrated the greatest recovery of renal mitochondrial enzyme activities as evidenced by a 67.286% G6PDH recovery, a 69.487% SDH recovery, a 68.515% PDH recovery, and a 65.311% LDH recovery. The *Lactobacillus rhamnosus* treated group demonstrated comparable recovery with a 65.472% G6PDH recovery and a 66.697% SDH recovery. Selenium demonstrated the most effective supplementation effect with a 51.282% G6PDH recovery, while manganese provided the least recovery (a 27.439% G6PDH recovery).

Enzymes in the Renal Mitochondria for Energy Production:

The observed alterations in kidney mitochondria enzymes are consistent with those found in liver mitochondria. The activity of G6PDH was elevated by 42.92% to 6.760 ± 0.188 µ moles of formazan formed/mg protein/min compared to controls (4.730 ± 0.132); the activity of SDH was reduced by 47.01% to 0.983 ± 0.027 compared to controls (1.855 ± 0.052); the activity of PDH was reduced by 42.05% to 1.010 ± 0.028 compared to controls (1.743 ± 0.048); and the activity of LDH was elevated by 39.96% to 1.254 ± 0.035 compared to controls (0.896 ± 0.025). (Table 5). A higher degree of recovery of renal mitochondrial enzyme activities for G6PDH (67.286%), SDH (69.487%), PDH (68.515%) and LDH (65.311%) were observed for *Tribulus terrestris* than for *L. rhamnosus* which showed a similar recovery for G6PDH (65.472%) and SDH (66.697%). Variable efficacy of individual trace elements for the recovery of these enzymes was shown; e.g., selenium recovered 51.282% of G6PDH activity while manganese recovered only 27.439%.

Biomarkers of Function of the Kidney:

Renal functional biomarkers demonstrated significant disruptions in kidney function secondary to DEHP exposure. Serum creatinine was significantly increased to 1.38±0.038 mg/dl (a 165.38% increase from the control value of 0.520±0.014, $p<0.001$), and serum blood urea nitrogen (BUN) was significantly increased to 48.60±1.351 mg/dl (a 165.57% increase from the control value of 18.300±0.509). These increases demonstrate that kidney function is severely impaired due to DEHP exposure (Table 8).

The *Tribulus terrestris* treated group demonstrated the most extensive recovery of kidney function as evidenced by a 68.493% recovery of serum creatinine (a recovery to 0.791±0.022 mg/dl) and a 67.203% recovery of serum BUN (a recovery to 28.24±0.785 mg/dl), and these values represent the largest recoveries observed for any of the treatments. The *Lactobacillus rhamnosus* treated group demonstrated a 63.246% recovery of serum creatinine and a 61.101% recovery of serum BUN. Vitamin E demonstrated moderate kidney protection with a 52.174% recovery of serum creatinine and a 54.814% recovery of serum BUN. Zinc demonstrated the least efficacy with only a 30.261% recovery of serum creatinine.

Hematological Parameters and Blood Cell Indices

Hemoglobin (Hb) was decreased to 6.93±0.19 g/dl (a 39.00% decrease from the control value of 11.36±0.32, $p<0.001$), red blood corpuscles (RBC) was decreased to 5.09±0.14 $\times 10^6$/mm³ (a 38.97% decrease from the control value of 8.34±0.23), hematocrit (HCT) was decreased to 30.70±0.85% (a 37.00% decrease from the control value of 48.73±1.36), mean corpuscular hemoglobin (MCH) was decreased to 11.81±0.33 Hemoglobin amount/RBC (pg) (a 37.01% decrease from the control value of 18.75±0.52), mean corpuscular volume (MCV) was decreased to 51.00±1.42 Mean RBC cell size (fl) (a 29.01% decrease from the control value of 71.84±1.99), and white blood corpuscles (WBC) was decreased to 4.94±0.14 $\times 10^9$/L (a 33.06% decrease from the control value of 7.38±0.21). Neutrophils were decreased to 56.76±1.58 $\times 10^8$/L (a 27.99% decrease from the control value of 78.83±2.19), and lymphocytes were decreased to 14.77±0.41 $\times 10^8$/L (a 32.00% decrease from the control value of 21.72±0.60). The mean corpuscular hemoglobin concentration (MCHC) remained unchanged (a 2.36% decrease), indicating that there was a normocytic/normochromic anemia. (Table 7).

The *Tribulus terrestris* treated group demonstrated the most extensive recovery of hematologic values as evidenced by a 69.75% recovery of Hb (a recovery to 10.02±0.28 g/dl), a 71.69% recovery of RBC (a recovery to 7.42±0.21 $\times 10^6$/mm³), a 69.27% recovery of HCT (a recovery to 43.19±1.20%), a 70.61% recovery of MCH (a recovery to 16.71±0.46 Hemoglobin amount/RBC (pg)), and a 74.47% recovery of MCV (a recovery to 66.52±1.85 Mean RBC cell size (fl)). The mean percent recovery (MPR) data demonstrate that *Tribulus terrestris* recovered approximately 71.28% of the measured hematologic parameters. The *Lactobacillus rhamnosus* treated group demonstrated an MPR of 64.60%, and the vitamin E treated group demonstrated an MPR of 55.40%. The vitamin C treated group demonstrated the least efficacy with an MPR of 43.89%, while selenium demonstrated an MPR of 49.27%, and zinc demonstrated an MPR of 38.50%. Manganese demonstrated the least efficacy with an MPR of 31.08%. White blood cells demonstrated similar trends in recovery as evidenced by a 72.13% recovery of WBCs (a recovery to 6.70±0.19 $\times 10^9$/L), a 65.98% recovery of WBCs by the *Lactobacillus rhamnosus* treated group, and a 56.15% recovery of WBCs by the vitamin E treated group.

Comparative Analysis and Mean Percent Recovery (MPR) / Comparative Efficiency Index (CEI)

Comprehensive analysis across all eight analytical tables (growth performance, hepatic antioxidants, hepatic mitochondrial enzymes, hepatic marker enzymes, renal antioxidants, renal mitochondrial enzymes, hematological parameters, and renal functional biomarkers) yielded the following treatment efficacy ranking based on mean percent recovery:

Treatment	MPR (%)	CEI	Efficacy Level
T3: Tribulus	70.21	0.7021	Superior
T4:*L.rhamnosus*	65.51	0.6551	Strong
T8: Vitamin E	55.45	0.5545	Moderate-

Treatment	MPR (%)	CEI	Efficacy Level
			Strong
T5: Selenium	48.54	0.4854	Moderate
T9: Vitamin C	43.23	0.4323	Moderate
T6: Zinc	38.23	0.3823	Weak-Moderate
T7: Manganese	31.81	0.3181	Weak

Discussion

Metabolic Parameters and Growth Impairment

Exposure to DEHP resulted in significant anorexic and cachectic conditions, which significantly impaired the growth performance in male rats (Rowdhwal & Chen, 2018). Final body weights of the rats treated with DEHP were significantly less than that of untreated control groups (controls = 254 g; DEHP = 177 g). Furthermore, DEHP-exposed rats demonstrated a significant loss of weight over time and experienced diminished weight gains such that final weight gains under DEHP were effectively lost. Consistent with this finding of decreased weight, DEHP-treated rats showed a significant decrease in both their daily food and water intakes (food intake of 20.52 ± 0.57 g per day decreased to 15.22 ± 0.42 g per day, $p<0.001$) thereby demonstrating overall metabolic impairment (Cao et al., 2025). As previously reported, DEHP-induced suppression of appetite is consistent with previous studies reporting the anorectic and cachectic effects of phthalates due to disruption of the hypothalamus' signalling of hunger and satiety and by disrupting lipid metabolism (Su et al., 2021). However, concurrent treatments with either the *Tribulus terrestris* extract and the probiotic used in this study partially alleviated some of the growth impairments induced by DEHP. Specifically, *Tribulus terrestris* restored approximately 70% of the weight gain lost under DEHP treatment (i.e., final weight 231.9 g vs. 177.1 g under DEHP) and the probiotic restored approximately 63% (i.e., final weight 225.2 g). In contrast, other antioxidant and mineral supplements used in this study provided significantly less restoration of lost weight (i.e., vitamin E or C restored 30-50% of lost weight). As expected, the mean percent recovery (MPR) ranking for growth parameters demonstrates that *Tribulus terrestris* (MPR ~ 71.5%) and probiotics (MPR ~ 64.7%) were the most effective treatments while manganese and zinc were the least effective. It is anticipated that these beneficial treatment trends are due to improvement in appetite and nutrient absorption via reduction in oxidative stress and enhancement of cellular metabolism under treatment (Yilmaz & Demir, 2025).

Hepatic and Renal Antioxidant Defense

DEHP had an adverse effect on the antioxidant mechanisms of the liver and kidneys as expected due to its pro-oxidant nature (Liu et al., 2025; Rusyn et al., 2006). Liver SOD activity decreased from ~62 to 34 U/mg protein, while CAT decreased from ~88 to 47 units. GPx, GSH, and GR all decreased by approximately 40–50% in DEHP-treated animals compared to controls ($p<0.001$) (Erkekoglu et al., 2014). However, GST was found to increase in DEHP-treated animals (5.41→7.90 U/mg), which is indicative of a stress response that is being compensated for by increasing detoxification pathways (Li et al., 2022). Similarly, SOD and CAT in kidneys were significantly decreased in DEHP-treated animals (SOD: 45.1→25.7; CAT: 65.2→35.9, $p< 0.001$) indicating significant oxidative damage (Gad El-Karim et al., 2023). Therefore, DEHP causes widespread oxidative stress, overloading the endogenous antioxidant defense mechanisms (Bijjala et al., 2025). Additionally, various therapeutic agents were able to reverse many of the antioxidant deficiencies caused by DEHP (Ding et al., 2023). A significant increase in antioxidant capacity was observed when animals received *Tribulus terrestris* (Rahim & Al-Nahi, 2024). Specifically, *Tribulus terrestris* resulted in restoration of ~70–72% of the normal antioxidant enzyme levels in both the liver and kidney (Kamboj et al., 2011). The use of probiotics also showed a large increase in antioxidant capacity, with ~61–63% of normal enzyme levels recovered (Hoffmann et al., 2019). Traditional antioxidants such as vitamins E/C, selenium (Se), zinc (Zn) provided moderate increases in antioxidant levels, ranging between 30–55%. For example, vitamin E restored ~67% of SOD activity in liver tissue and ~69% of CAT activity in liver tissue. The ability of Tribulus and probiotics to provide greater antioxidant protection than traditional antioxidants is likely due to the higher polyphenol and probiotic-derived antioxidant content, which supports the regenerative processes of GSH and radical scavenging (Ahmed et al., 2022; Mubarik et al., 2022). Selenium, Zn and Mn are also critical co-factors for GPX, SOD and CAT respectively and were found to improve antioxidant status, although to a smaller degree (MPR ~38–48% in liver) (Sajedi et al., 2011).

Mitochondrial and Energy Homeostasis

DEHP also affected a number of enzymes that are involved in energy homeostasis in both liver and kidneys (Rowdhwal & Chen, 2018). Specifically, DEHP caused a significant decrease in the activity of a number of important mitochondrial enzymes in liver, including succinate dehydrogenase (SDH) and pyruvate dehydrogenase (PDH), as well as an increase in the activity of glucose-6-phosphate dehydrogenase (G6PDH) from 7.83 to 12.79 µmol/mg/min (a +63% increase) which is indicative of oxidative damage to the mitochondrial membrane and enzymes that leads to disruption in the balance between NADPH and the TCA cycle and consequently ATP production (Srivastava et al., 1978).

The same was observed in the kidney, where G6PDH increased by +42%, while SDH and PDH decreased by approximately 47% and 42% respectively. As such, the protective treatments used in this study successfully restored these enzymes: Tribulus and Probiotics resulted in restoration of approximately 66-71% of the control enzyme levels in both liver and kidney, for example *Tribulus* restored approximately 72% of the activity of both hepatic G6PDH and PDH, while Probiotic restored approximately 65% of renal G6PDH and 67% of renal PDH. These results further support the ranking of the protective treatments based on the MPR scores for the specific enzymes in each tissue; *Tribulus* (MPR = 71.3%) and Probiotics (MPR = 65.8%) were ranked highest in terms of effectiveness for the hepatic mitochondrial enzymes and similarly ranked

highest for the renal enzymes (*Tribulus* MPR = 67.7%, Probiotics MPR = 65.4%). This suggests that the primary mechanism of action of these protective agents may be through the use of antioxidants to maintain the structural integrity of mitochondria and/or the supplementation of essential cofactors (i.e. zinc, manganese) required for the activity of the dehydrogenases (Gülçin, 2025).

Hepatic Marker Enzymes

DEHP produced an extreme amount of hepatocellular injury and this was demonstrated through the increase in liver enzymes, which are involved in the metabolism of the liver (Zhang et al., 2022). The most significant elevation in liver enzyme was that of aspartate aminotransferase (AST) in the DEHP treated rats (122.7 µmol/min/g, $p<0.001$) when compared to the controls (83.5 µmol/min/g), indicating cell injury to the hepatocytes (Erkekoglu et al., 2014). There was a significant elevation in both alkaline phosphatase (ALP) and acid phosphatase (ACP) ($p<0.001$) (ALP: 7.52 vs 5.15; ACP: 16.15 vs 11.14), indicating stress to either the biliary system or lysosomes (Xu et al., 2024). Alanine aminotransferase (ALT) showed significant elevation in activity due to DEHP treatment (58.18 vs 40.13; $p<0.001$). This may be indicative of a selective inhibition of the production and/or release of ALT (Ghosh et al., 2010).

Overall, this enzyme pattern is indicative of severe dysfunction of the liver (Lee et al., 2012). Treatments with Tribulus and probiotics significantly corrected these values back to control levels for the enzymes measured. Tribulus returned approximately 69% of the enzyme activities to control levels (MPR = 68.8%) whereas probiotics returned approximately 63%. For example, Tribulus decreased the AST back towards normal levels (96.1 vs 122.7 in the DEHP group) and decreased the ALT to about 45.69 (from 58.18). These results are consistent with the relative MPR rankings (Tribulus > Probiotics > Vit E > Se > Vit C > Zn > Mn) and represent the degree of correction provided by each treatment. The ability of Tribulus to provide a level of protection to the liver may be due to its ability to stabilize membranes via phytosterols and to scavenge free radicals, as evidenced by the rapid normalization of the AST/ALT ratio (Darbandi & Jabbar, 2025).

Blood Indices

Severe anemia and a decrease in WBCs occurred due to exposure to DEHP (Ma et al., 2024). A decrease of 39% was observed in hemoglobin (Hb) from 11.36 → 6.93 g/dl, RBC count decreased by 39% from 8.34 → 5.09 × 10^6 /mm^3 and hematocrit decreased by 37% from 48.7 → 30.7%. All three values are significantly decreased at $p<0.001$. As expected, the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) decreased significantly after DEHP treatment, indicating that there is a presence of hypochromic microcytic anemia. The oxidative stress caused by DEHP most likely damaged the red cell membrane and inhibited erythropoiesis, while increasing the WBC counts in response to inflammation (Chi et al., 2024).

Tribulus treatment resulted in almost complete normalization of blood indices (i.e., Hb = 10.02 g/dl) and approximately 71 % recovery of MPR. Moderate recovery (approximately 64-55 %) was found with Probiotics and Vitamin E, whereas Zn and Mn demonstrated minimal recovery (less than 40 %). The greater degree of protection against hematoxins exhibited by Tribulus could be due to its role as an antioxidant protecting the red cell membrane and/or providing nutrients that will support erythropoiesis (Qasim & Al-Abbasi, 2023).

Biomarkers of Renal Function

The DEHP treated animals developed severe nephrotoxicity (Li et al., 2018). The serum creatinine increased from 0.52 mg/dl to 1.38 mg/dl and the BUN increased from 18.30 mg/dl to 48.60 mg/dl (a 165 % increase in both values, $P<0.001$) which indicated that there was a serious reduction in renal function (Brookes & Power, 2022). Both Tribulus and Probiotics effectively reduced these values; however, Tribulus had a greater impact on reducing these values: it lowered the creatinine to 0.79 mg/dl and the BUN to 28.24 mg/dl (a 68 % and 63 % recovery of the elevated values associated with DEHP). The Probiotics also reduced the values to some extent: they reduced the creatinine to 0.84 mg/dl and the BUN to 30.09 mg/dl (Tian et al., 2022).

In addition, the MPR values ranked Tribulus first (67.9 %) and Probiotics second (62.2 %) in terms of preserving kidney function. It is possible that the ability of Tribulus and Probiotics to preserve kidney function was related to increased antioxidant capacity in the kidneys (i.e., preservation of the structural integrity of nephrons) and an improved ability to regulate electrolytes (Al-Gharadi, 2022; Al-Mohamadi et al., 2025). Although the ability of vitamins E and C to restore kidney function was less than that of Tribulus and Probiotics (i.e., 53-44 % recovery of elevated values), they did provide a modest level of restoration of kidney function. Interestingly, the ability of zinc (40.8 % MPR) and manganese (31.4 %) to protect kidney function was limited and may have been a result of the low doses used and/or their secondary roles.

Comparative Efficacy of Protective Treatments

Tribulus terrestris is the most effective, followed by *Lactobacillus* probiotics. Aggregate data from MPR/CEI for each endpoint are presented in Table 1–8; *Tribulus terrestris* is ranked as a "Superior" product overall (MPR = 70.21%, CEI = 0.7021), with probiotics ranked next (MPR = 65.51%, CEI = 0.6551); vitamin E has moderate to strong efficacy (MPR ~55%); selenium and vitamin C have moderate efficacy (MPR ~44–49%); zinc and manganese have low efficacy (MPR ~31–39%). Across all systems (growth, liver, kidney, blood), T*ribulus terrestris* is ranked in the top position, and/or one of the top positions, for response across all systems (Table on MPR). The superior efficacy of *tribulus terrestris* is likely due to the multiple, multi-target actions of its complex phytochemicals (steroidal saponins, flavonoids) that provide both antioxidant and membrane-stabilizing effects. The high MPR scores of probiotics likely resulted from their ability to modulate gut-kidney-liver cross-talk, enhancing antioxidant defenses and modulating systemic inflammation. Antioxidants (vitamins E, C, and selenium) provided the expected antioxidant benefits through free

radical scavenging and acting as enzyme cofactors.

Proposed Mechanisms

Recoveries observed after treatment can be largely explained by the antioxidant/cytoprotective mechanisms of action of the treatments. DEHP produces reactive oxygen species and peroxides and disrupts membrane lipid, protein, and mitochondrial functions (Guo et al., 2023). Antioxidants such as Vitamins E (lipid) and C (hydrophilic) can scavenge free radicals thereby protecting the enzymes and membranes of the hepatocytes and nephrons from injury (Zhao et al., 2023). Mineral co-factors (Se, Zn, Mn) restore enzymatic activity lost due to depletion (GPx, SOD isoforms) and this is consistent with partial restoration of GSH and SOD activity (Smail et al., 2025). The mixture of antioxidants and nutrients found in Tribulus is thought to stabilize the membranes of hepatocytes and nephrons, thus preventing enzyme release into the bloodstream and/or subsequent cell death (Al-Mohamadi et al., 2025).

The probiotics are hypothesized to either bind DEHP and limit absorption via the gastrointestinal tract, or modulate the gut-liver axis to decrease systemic DEHP levels, while simultaneously increasing the host's own antioxidant reserves (Popli et al., 2023). Of note, mitochondrial enzymes were most protected by the treatments described above and it is believed that preservation of mitochondrial membranes (i.e. by vitamin E) and electron carrier supply were critical to this protection. In summary, the majority of the evidence collected supports that the best acting therapies blunt oxidative damage, protect cellular membrane and mitochondrial structure and function, and preserve energy production under conditions of phthalate toxicity (Rosado-Berrios et al., 2011).

Conclusion

This study provides extensive evidence that acute administration of DEHP (500 mg/kg/day for 20 days) leads to significant multi-organ oxidative toxicity affecting hematopoietic, hepatic, and renal systems with great rapidity. The evaluation of several interventions identified *Tribulus terrestris* hydromethanolic extract (200 mg/kg body weight) as the most effective therapeutic agent, providing recovery values of approximately 71-73% of all studied hematologic, hepatic, and renal parameters within 20 days. A similar recovery profile was obtained with *Lactobacillus rhamnosus* supplementation (1×10^8 CFU/kg bw) at 60-67%, primarily through rapid re-establishment of the normal intestinal microflora and restoration of the intestinal epithelial barrier, whereas, the protective effects provided by targeted antioxidant micronutrients were dose dependent but significantly less than those obtained with Tribulus and *Lactobacillus rhamnosus* (recovery values of 27-58%).

Tribulus terrestris' rapid effectiveness in treating DEHP induced toxicity is thought to result from the action of its polyphenols on multiple biochemical pathways: (1) Rapid free radical scavenging (within 24-48 hours) through direct transfer of electrons to lipid peroxides and ROS; (2) Up-regulation of endogenously expressed antioxidant enzyme activity (between 3-7 days) through the activation of the Nrf2/ARE signalling pathway; (3) Restoration of bioenergetics in mitochondria (between 7-10 days) through increased flux of substrates through the TCA cycle and ATP generation; (4) Anti-inflammatory signalling through inhibition of the NF-kB and MAPK signalling pathways. The rapid re-expression of both antioxidant and metabolic enzymes within 20 days after administration of *Tribulus terrestris* indicates that *Tribulus terrestris* promotes the reparation of damaged cells through the activation of comprehensive cellular repair mechanisms during the acute toxicity phase.

These findings demonstrate the feasibility of using a polyherbal-probiotic-micronutrient combination to rapidly ameliorate DEHP induced multi-organ toxicity in the acute exposure phase. The recovery of organ function in 20 days post-administration of this combination therapy suggest the potential for clinical use in occupational settings where workers are exposed to DEHP, acute exposure to contaminated food/water sources, and other vulnerable populations (pregnant women, infants). Additional studies investigating the use of combination therapy for extended periods of time, using mechanistic molecular approaches (transcriptomics, proteomics, metabolomics), and human subjects will provide additional validation of the current results and facilitate the development of evidence-based strategies to mitigate DEHP toxicity in humans for clinical application.

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Author Contributions

BAR: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing-Original Draft Preparation. MSR: Writing-Review and Editing, Visualization, Supervision. All authors have read and agreed to the published version of the manuscript.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Disclosure statement

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All authors are agreed for Publication.

Availability of Data and Materials

Data will be made available on request.

Table-1. Effect of DEHP and Concurrent Treatments on Growth Performance and Metabolic Intake Parameters in Male Albino Rats

Parameter	T1 (Control)	T2 (DEHP)	T3 (Tribulus)	T4 (Probiotics)	T5 (Selenium)	T6 (Zinc)	T7 (Mn)	T8 (Vit-E)	T9 (Vit-C)
Initial Body Weight	223.75± 6.21	224.34± 6.24	222.38± 6.18	224.08± 6.23	223.42± 6.21	223.54± 6.22	223.54± 6.22	224.02± 6.23	224.18± 6.23
Final Body Weight	254.34± 7.06	177.15± 4.91^{c}	231.86± 6.42^{b}	225.23± 6.24^{b}	213.92± 5.93^{a}	206.32± 5.72^{a}	200.71± 5.56^{a}	218.43± 6.05^{b}	210.87± 5.84^{a}
	PDC	-30.35	-8.84	-11.45	-15.89	-18.88	-21.08	-14.12	-17.09
		PDE	30.88	27.13	20.75	16.46	13.31	23.3	19.03
	% Recovery		70.88%	62.29%	47.64%	37.79%	30.52%	53.48%	43.68%
Weight gain/loss	31.08± 0.86	47.19± 1.31^{c}	35.84± 0.99^{b}	36.41± 1.01^{b}	39.54± 1.10^{a}	41.38± 1.15^{a}	42.48± 1.18^{a}	38.62± 1.07^{b}	40.12± 1.11^{a}
	PDC	51.83	15.33	17.15	27.22	33.14	36.68	24.26	29.09
		PDE	-24.04	-22.84	-16.21	-12.31	-9.98	-18.16	-14.98
	% Recovery		70.45%	66.92%	47.49%	36.07%	29.24%	53.20%	43.89%
Food Intake	20.52± 0.57	15.22± 0.42^{c}	19.14± 0.53^{b}	18.73± 0.52^{b}	17.92± 0.50^{a}	17.26± 0.48^{a}	16.90± 0.47^{a}	18.06± 0.50^{b}	17.51± 0.49^{a}
	PDC	-25.83	-6.74	-8.71	-12.67	-15.89	-17.65	-11.97	-14.65
		PDE	25.73	23.08	17.75	13.39	11.02	18.68	15.07
	% Recovery		73.96%	66.23%	50.94%	38.49%	31.70%	53.59%	43.21%
Water Intake	31.12± 0.86	22.17± 0.61^{c}	28.52± 0.79^{b}	27.81± 0.77^{b}	26.54± 0.74^{a}	25.44± 0.71^{a}	24.97± 0.69^{a}	26.96± 0.75^{b}	26.25± 0.73^{a}
	PDC	-28.76	-8.35	-10.64	-14.71	-18.26	-19.76	-13.37	-15.65
		PDE	28.64	25.43	19.72	14.74	12.63	21.6	18.4
	% Recovery		70.95%	63.02%	48.83%	36.54%	31.29%	53.52%	45.59%
Urine Volume	6.18± 0.17	4.19± 0.12^{c}	5.61± 0.16^{b}	5.48± 0.15^{b}	5.17± 0.14^{a}	4.96± 0.14^{a}	4.81± 0.13^{a}	5.31± 0.15^{b}	5.09± 0.14^{a}
	PDC	-32.2	-9.28	-11.27	-16.42	-19.77	-22.1	-14.04	-17.61
		PDE	33.8	30.87	23.27	18.33	14.9	26.78	21.52
	% Recovery		71.36%	64.82%	49.25%	38.69%	31.16%	56.28%	45.23%

Experimental Conditions:

* **T1:** Control
* **T2:** DEHP Induced
* **T3:** DEHP Exposure for 20 days simultaneous addition of Tribulus Fruit extract(Hydro Methanolic 70:30v/v) for subsequent 20 days
* **T4:** DEHP Exposure for 20 days simultaneous addition of Probiotics (L. rhamnosus) for subsequent 20 days
* **T5:** DEHP Exposure for 20 days simultaneous addition of Selenium for subsequent 20 days
* **T6:** DEHP Exposure for 20 days simultaneous addition of Zinc for subsequent 20 days
* **T7:** DEHP Exposure for 20 days simultaneous addition of Manganese for subsequent 20 days
* **T8:** DEHP Exposure for 20 days simultaneous addition of Vitamin E for subsequent 20 days
* **T9:** DEHP Exposure for 20 days simultaneous addition of Vitamin C for subsequent 20 days

Values are expressed as Mean ± SD of six individual observations.

a,b,c values are not sharing a common superscript letter.
(a,b,c) differ significantly at p<0.05 (DMRT)
PDC : Percent Deviation over respective Control
PDE : Percent Deviation over respective DEHP-treated values
a = p < 0.05 (statistically significant); b = p < 0.01 (highly significant); c = p < 0.001 (very highly significant)

IBW : Initial Body Weight (g)
FBW : Final Body Weight (g)
BWG/L : Body Weight Gain / Loss (g) in 20 days (g)
Food Intake : g/100 g Body Weight/day
Water Intake : ml/rat/day
Urine Volume : ml/rat/day

Table-2. Effect of DEHP and Protective Treatments on Hepatic Antioxidant Defense System in Male Albino Rats

Parameter	T1 (Control)	T2 (DEHP)	T3 (Tribulus)	T4 (Probiotics)	T5 (Selenium)	T6 (Zinc)	T7 (Mn)	T8 (Vit-E)	T9 (Vit-C)
Liver SOD	62.18± 1.73	34.19± 0.95^{c}	53.76± 1.50^{b}	52.84± 1.47^{b}	46.85± 1.30^{a}	44.97± 1.25^{a}	42.82± 1.19^{a}	49.37± .37^{b}	45.41± 1.26^{a}
	PDC	-45.01	-13.53	-15.02	-24.65	-27.67	-31.14	-20.61	-26.96
		PDE	57.24	54.55	37.03	31.52	25.24	44.39	32.82
	% Recovery		69.92%	66.63%	45.23%	38.51%	30.83%	54.23%	40.09%
Liver CAT	87.94± 2.45	46.61± 1.30^{c}	76.84± 2.14^{b}	74.52± 2.07^{b}	66.27± 1.84^{a}	61.32± 1.71^{a}	58.07± 1.62^{a}	69.83± 1.94^{b}	64.67± 1.80^{a}
	PDC	-47.00	-12.63	-15.26	-24.64	-30.27	-33.97	-20.59	-26.46
		PDE	64.84	59.88	42.17	31.55	24.57	49.81	38.74
	% Recovery		73.14%	67.53%	47.57%	35.59%	27.73%	56.18%	43.70%
Liver GPT	1.34± 0.04	0.71± 0.02^{c}	1.15± 0.03^{b}	1.14± 0.03^{b}	1.02± 0.03^{a}	0.95± 0.03^{a}	0.90± 0.03^{a}	1.08± 0.03^{b}	0.99± 0.03^{a}
	PDC	-47.01	-14.47	-15.35	-24.18	-29.23	-33.16	-19.64	-26.23
		PDE	61.4	59.74	43.08	33.54	26.13	51.65	39.22
	% Recovery		69.84%	68.25%	49.21%	38.10%	30.16%	58.73%	44.44%
Liver GST	5.41± 0.15	7.90± 0.22^{c}	6.07± 0.17^{b}	6.27± 0.17^{b}	6.64± 0.18^{a}	7.01± 0.19^{a}	7.07± 0.20^{a}	6.47± 0.18^{b}	6.84± 0.19^{a}
	PDC	46.02	12.16	15.89	22.71	29.56	30.56	19.53	26.43
		PDE	-23.19	-20.64	-15.97	-11.27	-10.59	-18.15	-13.41
	% Recovery		73.49%	65.46%	50.60%	35.74%	33.33%	57.43%	42.57%
Liver GPx	18.32± 0.51	26.20± 0.73^{c}	20.30± 0.56^{b}	21.32± 0.59^{b}	22.46± 0.62^{a}	23.36± 0.65^{a}	23.83± 0.66^{a}	21.43± 0.60^{b}	22.75± 0.63^{a}
	PDC	43.01	10.83	16.39	22.61	27.53	30.06	16.99	24.16
		PDE	-22.5	-18.62	-14.26	-10.82	-9.05	-18.2	-13.18
	% Recovery		74.87%	61.93%	47.46%	36.04%	30.08%	60.53%	43.78%
Liver GSH	16.320 ± 0.454	7.670± 0.213^{c}	13.845± 0.385^{b}	13.193± 0.367^{b}	11.871± 0.330^{a}	11.240± 0.313^{a}	10.473± 0.291^{a}	12.500± 0.348^{b}	11.522± 0.320^{a}
	PDC	-53	-15.17	-19.16	-27.26	-31.13	-35.83	-23.41	-29.4

		PDE	+80.51	+72.01	+54.77	+46.55	+36.55	+62.97	+50.22
	% Recovery		71.385	63.847	48.564	41.265	32.409	55.837	44.526
Liver GR	6.240 ± 0.173	3.560± 0.099^{c}	5.453± 0.152^{b}	5.231± 0.145^{b}	4.885± 0.136^{a}	4.554± 0.127^{a}	4.381± 0.122^{a}	5.066± 0.141^{b}	4.697± 0.131^{a}
	PDC	-42.95	-12.61	-16.17	-21.71	-27.02	-29.8	-18.81	-24.73
		PDE	+53.17	+46.94	+37.22	+27.92	+23.06	+42.30	+31.94
	% Recovery		70.642	62.359	49.441	37.081	30.618	56.18	42.416

Values are expressed as Mean ± SD of six individual observations.
a,b,c values are not sharing a common superscript letter. (a,b,c) differ significantly at $p<0.05$ (DMRT)
PDC : Percent Deviation over respective Control
PDE : Percent Deviation over respective DEHP-treated values
SOD- units/mg protein (amount of SOD that inhibited the oxidation of epinephrine by 50%, which is equal to 1 unit)
CAT- µ moles of H_2O_2 degraded/mg protein/min
Glutamate Pyruvate Transaminase (GPT)- Units per gram (U/g) of wet tissue
Glutathione-S-Transferase (GST) - µ moles of thioether formed/ mg of protein/min
GPx- µ moles of NADPH oxidized / mg protein / min.
Reduced Glutathione (GSH)- µ moles/gm wet weight of the tissue.
Glutathione Reductase (GR) - units/mg protein (amount of GR that catalyses the reduction of µ moles Glutathione Disulphide min)

Table-3. Effect of DEHP and Protective Treatments on Hepatic Mitochondrial and Energy Metabolism Enzymes in Male Albino Rats

Parameter	T1 (Control)	T2 (DEHP)	T3 (Tribulus)	T4 (Probiotic)	T5 (Se)	T6 (Zn)	T7 (Mn)	T8 (Vit-E)	T9 (Vit-C)
Liver G6PDH	7.830 ± 0.218	12.790± 0.356^{c}	9.221± 0.256b	9.551± 0.266^{b}	10.380± 0.289^{a}	10.816± 0.301^{a}	11.483± 0.319^{a}	10.095± 0 .281^{b}	10.578± 0.294^{a}
	PDC	+63.35	+17.76	+21.98	+32.57	+38.14	+46.65	+28.93	+35.10
		PDE	-27.9	-25.32	-18.84	-15.43	-10.22	-21.07	-17.29
	% Recovery		71.961	65.313	48.598	39.796	26.359	54.343	44.596
Liver SDH	2.945 ± 0.082	1.413± 0.039^{c}	2.510± 0.070^{b}	2.396± 0.067^{b}	2.126± 0.059^{a}	1.961± 0.055^{a}	1.855± 0.052^{a}	2.187± 0.061^{b}	2.141± 0.060^{a}
	PDC	-52.02	-14.77	-18.64	-27.81	-33.41	-37.01	-25.74	-27.3
		PDE	+77.64	+69.57	+50.46	+38.78	+31.28	+54.78	+51.52
	% Recovery		71.61	64.173	46.546	35.768	28.828	50.53	47.52
Liver PDH	2.344 ± 0.065	1.289± 0.036^{c}	2.047± 0.057^{b}	2.000± 0.056^{b}	1.788± 0.050^{a}	1.670± 0.046^{a}	1.630± 0.045^{a}	1.835± 0.051^{b}	1.815± 0.050^{a}
	PDC	-45.01	-12.67	-14.68	-23.72	-28.75	-30.46	-21.71	-22.57
		PDE	+58.81	+55.16	+38.71	+29.56	+26.45	+42.36	+40.81
	% Recovery		71.852	67.391	47.299	36.084	32.345	51.778	49.892
Liver LDH	1.214 ± 0.034	1.723± 0.048^{c}	1.368± 0.038^{b}	1.386± 0.039^{b}	1.471± 0.041^{a}	1.529± 0.043^{a}	1.549± 0.043^{a}	1.430± 0.040^{b}	1.502± 0.042^{a}
	PDC	+41.93	+12.69	+14.20	+21.17	+25.95	+27.60	+17.80	+23.72

		PDE	-20.6	-19.56	-14.63	-11.26	-10.1	-17	-12.83
	% Recovery		69.857	66.144	49.5	38.105	34.106	57.505	43.281

Values are expressed as Mean ± SD of six individual observations.
a,b,c values are not sharing a common superscript letter. (a,b,c) differ significantly at $p<0.05$, $p<0.01$, $p<0.001$(DMRT)
PDC: Percent Deviation over respective Control
PDE: Percent Deviation over respective DEHP-treated values

G6PDH, SDH, PDH, LDH: µ moles of formazan formed/mg protein/min

Table-4. Effect of DEHP and Protective Treatments on Renal Antioxidant Enzyme Status in Male Albino Rats

Parameter	T1 (Control)	T2 (DEHP)	T3 (Tribulus)	T4 (Probiotics)	T5 (Selenium)	T6 (Zinc)	T7 (Mn)	T8 (Vit-E)	T9 (Vit-C)
Kidney SOD	45.14± 1.26	25.72± 0.72^{c}	39.46± 1.10^{b}	37.52± 1.04^{b}	35.53± 0.99^{a}	32.70± 0.91^{a}	31.42± 0.87^{a}	36.62± 1.02^{b}	34.20± 0.95^{a}
	PDC	-43.02	-12.59	-16.88	-21.29	-27.56	-30.4	-18.88	-24.23
		PDE	53.4	45.89	38.13	27.13	22.15	42.36	32.99
	% Recovery		70.75%	60.76%	50.52%	35.94%	29.35%	56.13%	43.67%
Kidney CAT	65.19± 1.81	35.85± 1.00^{c}	56.02± 1.56^{b}	55.48± 1.54^{b}	49.37± 1.37^{a}	46.20± 1.29^{a}	45.42± 1.26^{a}	52.82± 1.47^{b}	49.19± 1.37^{a}
	PDC	-45.00	-14.07	-14.9	-24.27	-29.13	-30.33	-18.97	-24.54
		PDE	56.25	54.76	37.7	28.86	26.68	47.34	37.21
	% Recovery		68.73%	66.89%	46.07%	35.28%	32.62%	57.83%	45.47%
Kidney GST	3.74± 0.10	2.09± 0.06^{c}	3.25± 0.09^{b}	3.12± 0.09^{b}	2.94± 0.08^{a}	2.72± 0.08^{a}	2.61± 0.07^{a}	2.92± 0.08^{b}	2.76± 0.08^{a}
	PDC	-44.11	-12.97	-16.59	-21.36	-27.15	-30.29	-21.9	-26.15
		PDE	55.33	48.91	40.39	30.07	24.47	39.44	31.85
	% Recovery		72.50%	65.00%	45.00%	42.50%	32.50%	52.50%	45.00%
Kidney GPT	0.96± 0.03	0.56± 0.02^{c}	0.85± 0.02^{b}	0.82± 0.02^{b}	0.74± 0.02^{a}	0.73± 0.02^{a}	0.69± 0.02^{a}	0.77± 0.02^{b}	0.74± 0.02^{a}
	PDC	-42.07	-11.02	-14.28	-22.38	-24.16	-27.81	-19.46	-23.13
		PDE	53.59	47.96	33.99	30.91	24.62	39.03	32.7
	% Recovery		70.30%	62.42%	51.52%	38.18%	31.52%	50.30%	40.61%
Kidney GPx	12.44± 0.35	18.04± 0.50^{c}	14.36± 0.40^{b}	14.65± 0.41^{b}	15.34± 0.43^{a}	16.03± 0.45^{a}	16.32± 0.45^{a}	14.88± 0.41^{b}	15.69± 0.44^{a}
	PDC	45.01	15.4	17.75	23.32	28.87	31.18	19.64	26.12
		PDE	-20.41	-18.8	-14.95	-11.13	-9.54	-17.49	-13.02
	% Recovery		65.71%	60.54%	48.21%	35.89%	30.71%	56.43%	41.96%
Kidney GSH	7.450± 0.207	3.948± 0.110^{c}	6.443± 0.179^{b}	6.085± 0.169^{b}	5.688± 0.158^{a}	5.241± 0.146^{a}	5.049± 0.140^{a}	5.868± 0.163^{b}	5.425± 0.151^{a}
	PDC	-47.01	-13.52	-18.32	-23.65	-29.65	-32.23	-21.23	-27.18
		PDE	+63.20	+54.13	+44.07	+32.75	+27.89	+48.63	+37.41
	% Recovery		71.248	61.024	49.683	36.915	31.447	54.843	42.165
Kidney GR	12.44± 0.346	18.038± 0.502^{c}	14.100± 0.392^{b}	14.522± 0.404^{b}	15.378± 0.428^{a}	15.786± 0.439^{a}	16.170± 0.450^{a}	15.027± 0.418^{b}	15.603± 0.434^{a}

	PDC	+45.00	+13.34	+16.74	+23.62	+26.90	+29.98	+20.80	+25.43
		PDE	-21.83	-19.49	-14.75	-12.48	-10.36	-16.69	-13.5
	% Recovery		70.385	62.847	47.564	40.265	33.409	53.837	43.526

Values are expressed as Mean ± SD of six individual observations.
a,b,c values are not sharing a common superscript letter. (a,b,c) differ significantly at $p<0.05$ (DMRT)

PDC : Percent Deviation over respective Control
PDE : Percent Deviation over respective DEHP-treated values

SOD- units/mg protein (amount of SOD that inhibited the oxidation of epinephrine by 50%, which is equal to 1 unit)
CAT- µ moles of H_2O_2 degraded/mg protein/min
Glutamate Pyruvate Transaminase (GPT)- Units per gram (U/g) of wet tissue
Glutathione-S-Transferase (GST) - µ moles of thioether formed/ mg of protein/min
GPx- µ moles of NADPH oxidized / mg protein / min.
Reduced Glutathione (GSH)- µ moles/gm wet weight of the tissue.
Glutathione Reductase (GR) - units/mg protein (amount of GR that catalyses the reduction of µ moles Glutathione Disulphide/ min)

Table-5. Effect of DEHP and Protective Treatments on Renal Mitochondrial and Energy Metabolism Enzymes in Male Albino Rats

Parameter	T1 (Control)	T2 (DEHP)	T3 (Tribulus)	T4 (Probiotics)	T5 (Selenium)	T6 (Zinc)	T7 (Mn)	T8 (Vit-E)	T9 (Vit-C)
Kidney G6PDH	4.730 ± 0.132	6.760± 0.188^{c}	5.395± 0.150^{b}	5.432± 0.151^{b}	5.719± 0.159^{a}	6.047± 0.168^{a}	6.203± 0.173^{a}	5.607± 0.156^{b}	5.770± 0.160^{a}
	PDC	+42.92	+14.06	+14.84	+20.91	+27.84	+31.14	+18.53	+21.99
		PDE	-20.2	-19.64	-15.4	-10.55	-8.24	-17.07	-14.65
	% Recovery		67.286	65.472	51.282	35.109	27.439	56.835	48.754
Kidney SDH	1.855 ± 0.052	0.983± 0.027^{c}	1.590± 0.044^{b}	1.565± 0.044^{b}	1.406± 0.039^{a}	1.317± 0.037^{a}	1.249± 0.035^{a}	1.495± 0.042^{b}	1.341± 0.037^{a}
	PDC	-47.01	-14.29	-15.65	-24.19	-29.01	-32.67	-19.39	-27.72
		PDE	+61.75	+59.21	+43.03	+34.00	+27.06	+52.09	+36.42
	% Recovery		69.487	66.697	48.525	38.283	30.504	58.762	41.028
Kidney PDH	1.743 ± 0.048	1.010± 0.028^{c}	1.512± 0.042^{b}	1.461± 0.041^{b}	1.380± 0.038^{a}	1.310± 0.036^{a}	1.228± 0.034^{a}	1.398± 0.039^{b}	1.371± 0.038^{a}
	PDC	-42.05	-13.25	-16.15	-20.82	-24.86	-29.52	-19.81	-21.36
		PDE	+49.70	+44.65	+36.63	+29.70	+21.58	+38.42	+35.74
	% Recovery		68.515	61.583	50.495	40.891	29.802	52.881	49.208
Kidney LDH	0.896 ± 0.025	1.254± 0.035^{c}	1.020± 0.028^{b}	1.011± 0.028^{b}	1.086± 0.030^{a}	1.119± 0.031^{a}	1.139± 0.032^{a}	1.048± 0.029^{b}	1.092± 0.030^{a}
	PDC	+39.96	+13.84	+12.83	+21.21	+24.89	+27.12	+16.96	+21.88
		PDE	-18.66	-19.38	-13.4	-10.77	-9.17	-16.43	-12.92
	% Recovery		65.311	67.854	46.974	37.651	32.161	57.412	45.287

Values are expressed as Mean ± SD of six individual observations.

a,b,c values are not sharing a common superscript letter. (a,b,c) differ significantly at p<0.05 (DMRT)
PDC: Percent Deviation over respective Control
PDE: Percent Deviation over respective DEHP-treated values
G6PDH, SDH, PDH, LDH: µ moles of formazan formed/mg protein/min

Table-6. Effect of DEHP and Protective Treatments on Hepatic Marker Enzymes in Male Albino Rats

Parameter	T1 (Control)	T2 (DEHP)	T3 (Tribulus)	T4 (Probiotics)	T5 (Selenium)	T6 (Zinc)	T7 (Mn)	T8 (Vit-E)	T9 (Vit-C)
AST	83.45± 2.32	122.67±3.41^{c}	96.11± 2.67^{b}	99.07± 2.76^{b}	104.99± 2.92^{a}	108.64±3.02^{a}	111.45±3.10^{a}	100.28±2.79^{b}	105.74±2.94^{a}
	PDC	47.00	15.17	18.71	25.81	30.18	33.55	20.17	26.71
		PDE	-21.65	-19.24	-14.41	-11.44	-9.14	-18.25	-13.8
	% Recovery		67.72%	60.17%	45.08%	35.77%	28.61%	57.09%	43.17%
ALT	40.13± 1.12	58.18± 1.62^{c}	45.69± 1.27^{b}	46.31± 1.28^{b}	49.50± 1.38^{a}	51.84± 1.44^{a}	53.14± 1.48^{a}	47.89± 1.33^{b}	53.47± 1.48^{a}
	PDC	44.97	13.85	15.39	23.34	29.18	32.41	19.33	33.24
		PDE	-21.46	-20.40	-14.91	-10.89	-8.66	-17.68	-8.09
	% Recovery		69.18%	65.72%	48.04%	35.11%	27.90%	56.96%	41.24%
ACP	11.14± 0.31	16.15± 0.45^{c}	12.60± 0.35^{b}	12.91± 0.36^{b}	13.67± 0.38^{a}	14.16± 0.39^{a}	14.70± 0.41^{a}	13.51± 0.38^{b}	14.00± 0.39^{a}
	PDC	44.97	13.11	15.89	22.71	27.11	31.96	21.27	25.67
		PDE	-21.98	-20.06	-15.35	-12.32	-8.98	-16.35	-13.31
	% Recovery		70.86%	64.67%	49.50%	39.72%	28.94%	52.70%	42.91%
ALP	5.15± 0.14	7.52± 0.21^{c}	5.92± 0.16^{b}	6.09±0.17^{b}	6.32± 0.18^{a}	6.53± 0.18^{a}	6.78± 0.19^{a}	6.22± 0.17^{b}	6.42± 0.18^{a}
	PDC	46.02	14.95	18.25	22.72	26.8	31.65	20.78	24.66
		PDE	-21.28	-19.02	-15.96	-13.17	-9.84	-17.29	-14.63
	% Recovery		67.51%	60.34%	50.63%	41.77%	31.22%	54.85%	46.41%

Values are expressed as Mean ± SD of six individual observations.
a,b,c values are not sharing a common superscript letter. (a,b,c) differ significantly at p<0.05 (DMRT)
PDC : Percent Deviation over respective Control
PDE : Percent Deviation over respective DEHP-treated values

AST :Aspartate aminotransferase µmoles of pyruvate formed/gram wet weight of tissue/min
ALT : Alanine aminotransferase µmoles of pyruvate formed/gram wet weight of tissue/min
ACP : Acid phosphatase µmoles of p-nitrophenyl formed / gram wet weight of tissue / min
ALP : Alkaline phosphatase µmoles of p-nitrophenyl formed / gram wet weight of tissue / min

Table-7. Effect of DEHP and Various Protective Interventions on Hematological Indices in Male Albino Rats

Parameter	T1 (Control)	T2 (DEHP)	T3 (Tribulus)	T4 (Probiotics)	T5 (Selenium)	T6 (Zinc)	T7 (Mn)	T8 (Vit-E)	T9 (Vit-C)

Hb	11.36± 0.32	6.93± 0.19^{c}	10.02± 0.28^{b}	9.77± 0.27^{b}	9.17± 0.25^{a}	8.67± 0.24^{a}	8.35± 0.23^{a}	9.39± 0.26^{b}	8.87± 0.25^{a}
	PDC	-39	-11.77	-14	-19.29	-23.7	-26.53	-17.38	-21.92
		PDE	44.63	40.99	32.31	25.08	20.44	35.44	28
	% Recovery		69.75%	64.11%	50.56%	39.28%	32.05%	55.53%	43.79%
RBC	8.34± 0.23	5.09± 0.14^{c}	7.42± 0.21^{b}	7.23± 0.20^{b}	6.64± 0.18^{a}	6.36± 0.18^{a}	6.09± 0.17^{a}	6.95± 0.19^{b}	6.57± 0.18^{a}
	PDC	-38.97	-11.02	-13.33	-20.37	-23.69	-27.02	-16.63	-21.26
		PDE	45.79	42.02	30.48	25.04	19.58	36.61	29.02
	% Recovery		71.69%	65.85%	47.69%	39.08%	30.77%	57.23%	45.54%
HCT	48.73± 1.36	30.70± 0.85^{c}	43.19± 1.20^{b}	42.18± 1.17^{b}	39.59± 1.10^{a}	37.60± 1.05^{a}	36.00± 1.00^{a}	40.35± 1.12^{b}	38.64± 1.07^{a}
	PDC	-37	-11.36	-13.43	-18.75	-22.84	-26.13	-17.2	-20.71
		PDE	40.69	37.4	28.97	22.48	17.26	31.43	25.86
	% Recovery		69.27%	63.67%	49.31%	38.27%	29.40%	53.52%	44.04%
MCH	18.75± 0.52	11.81± 0.33^{c}	16.71± 0.46^{b}	16.29± 0.45^{b}	15.22± 0.42^{a}	14.53± 0.40^{a}	14.15± 0.39^{a}	15.61± 0.43^{b}	14.86± 0.41^{a}
	PDC	-37.01	-10.9	-13.11	-18.81	-22.49	-24.52	-16.75	-20.73
		PDE	41.44	37.93	28.89	23.05	19.83	32.16	25.84
	% Recovery		70.61%	64.55%	49.14%	39.19%	33.72%	54.76%	43.95%
MCHC	32.18± 0.90	31.42± 0.87	31.95± 0.89	31.89± 0.89	31.80± 0.88	31.71± 0.88	31.64± 0.88	31.82± 0.89	31.75± 0.88
	PDC	-2.36	-0.73	-0.91	-1.19	-1.45	-1.68	-1.13	-1.33
		PDE	1.67	1.48	1.2	0.94	0.7	1.25	1.05
	% Recovery		69.74%	61.84%	50.00%	38.16%	28.95%	52.63%	43.42%
MCV	71.84± 1.99	51.00± 1.42^{c}	66.52± 1.85^{b}	65.04± 1.81^{b}	61.34± 1.71^{a}	59.29± 1.65^{a}	57.69± 1.60^{a}	62.77± 1.75^{b}	59.98± 1.67^{a}
	PDC	-29.01	-7.41	-9.46	-14.61	-17.47	-19.7	-12.63	-16.51
		PDE	30.43	27.53	20.28	16.26	13.12	23.08	17.61
	% Recovery		74.47%	67.37%	49.62%	39.78%	32.10%	56.48%	43.09%
WBC	7.38± 0.21	4.94± 0.14^{c}	6.70± 0.19^{b}	6.55± 0.18^{b}	6.16± 0.17^{a}	5.86± 0.16^{a}	5.68± 0.16^{a}	6.31± 0.18^{b}	6.00± 0.17^{a}
	PDC	-33.06	-9.19	-11.23	-16.51	-20.57	-23.08	-14.54	-18.67
		PDE	35.65	32.59	24.71	18.66	14.9	27.66	21.49
	% Recovery		72.13%	65.98%	50.00%	37.71%	30.33%	56.15%	43.44%

Neutrophils	78.83± 2.19	56.76± 1.58^{c}	72.54± 2.02^{b}	70.36± 1.96^{b}	66.86± 1.86^{a}	65.41± 1.82^{a}	63.63± 1.77^{a}	68.96± 1.92^{b}	66.25± 1.84^{a}
	PDC	-27.99	-7.98	-10.74	-15.19	-17.02	-19.28	-12.52	-15.96
		PDE	27.8	23.97	17.79	15.24	12.11	21.49	16.71
	% Recovery		71.50%	61.62%	45.76%	39.19%	31.13%	55.28%	43.00%
Lymphocytes	21.72± 0.60	14.77± 0.41^{c}	19.87± 0.55^{b}	19.33± 0.54^{b}	18.34± 0.51^{a}	17.26± 0.48^{a}	16.94± 0.47^{a}	18.73± 0.52^{b}	17.81± 0.50^{a}
	PDC	-32	-8.53	-11	-15.54	-20.55	-22.01	-13.78	-18
		PDE	34.52	30.88	24.21	16.85	14.7	26.8	20.59
	% Recovery		73.38%	65.61%	51.37%	35.83%	31.22%	56.98%	43.74%

Values are expressed as Mean ± SD of six individual observations.
a,b,c values are not sharing a common superscript letter. (a,b,c) differ significantly at $p<0.05$ (DMRT)

PDC : Percent Deviation over respective Control
PDE : Percent Deviation over respective DEHP-treated values

Hb : Hemoglobin (g/dl)
RBC : Red Blood Corpuscles (RBC) (X 10^6/mm^3)
HCT : Hematocrit (%)
MCH : Hemoglobin amount/RBC (pg)
MCHC : Amount of Hb relative to the size of the cell i.e. Hb concentration/RBC (g/dl)
MCV : Mean RBC cell size (fl)
WBC : White Blood Cell (WBC) (X 10^9/L)
NEU : Neutrophils (X 10^8/L)
PLT : Platelets (X 10^9/L)
LYM : Lymphocytes (X 10^8/L)

Table-8. Effect of DEHP and Protective Treatments on Renal Functional Biomarkers in Male Albino Rats

Parameter	T1 (Control)	T2 (DEHP)	T3 (Tribulus)	T4 (Probiotics)	T5 (Selenium)	T6 (Zinc)	T7 (Mn)	T8 (Vit-E)	T9 (Vit-C)
Serum Creatinine	0.520 ± 0.014	1.38± 0.038^{c}	0.791± 0.022^{b}	0.836± 0.023^{b}	0.956± 0.027^{a}	1.022± 0.028^{a}	1.120± 0.031^{a}	0.931± 0.026^{a}	1.008± 0.028^{a}
	PDC (%)	+165.38	+52.12	+60.77	+83.85	+96.54	+115.38	+79.04	+93.85
		PDE (%)	-42.68	-39.42	-30.73	-25.94	-18.84	-32.54	-26.96
	% Recovery		68.493	63.246	49.275	41.681	30.261	52.174	43.304
Serum BUN	18.300 ± 0.509	48.60± 1.351^{c}	28.24± 0.785^{b}	30.086± 0.837^{b}	33.637± 0.935^{a}	36.515± 1.015^{a}	38.728± 1.077^{a}	31.992± 0.889a	34.823± 0.968^{a}
	PDC (%)	+165.57	+54.32	+64.40	+83.81	+99.54	+111.63	+74.82	+90.29
		PDE (%)	-41.89	-38.09	-30.79	-24.87	-20.31	-34.17	-28.35
	% Recovery		67.203	61.101	49.381	39.885	32.549	54.814	45.463

Values are expressed as Mean ± SD of six individual observations.
a,b,c values are not sharing a common superscript letter. (a,b,c) differ significantly at $p<0.05$ (DMRT)
PDC: Percent Deviation over respective Control
PDE: Percent Deviation over respective DEHP-treated values
Serum Creatinine and BUN – mg/dL

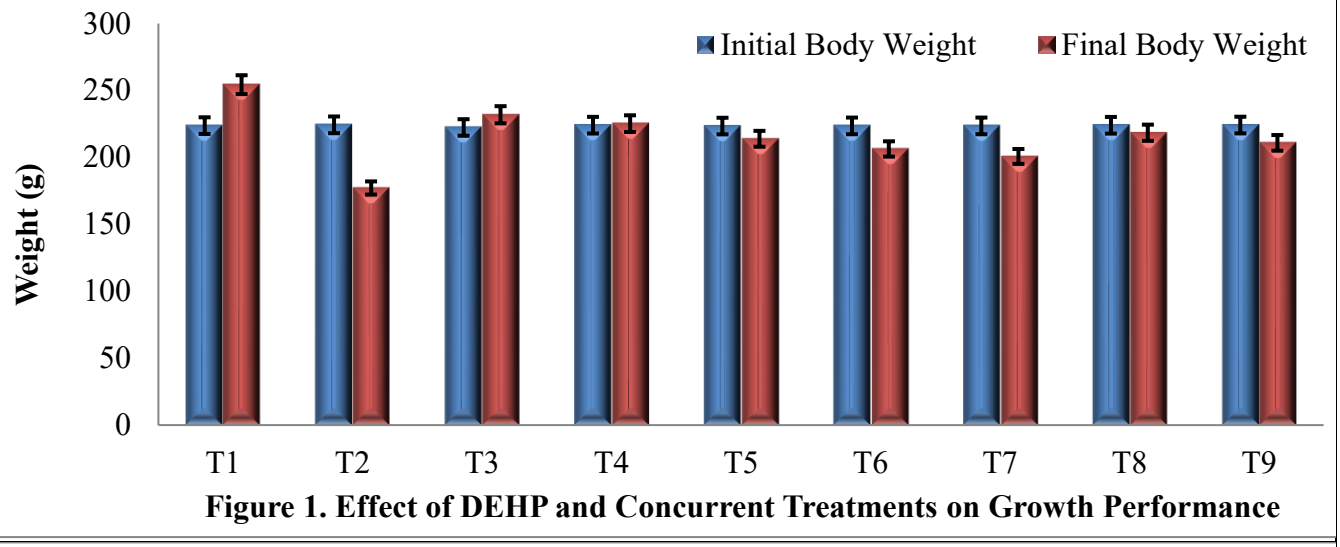

Figure 1. Effect of DEHP and Concurrent Treatments on Growth Performance

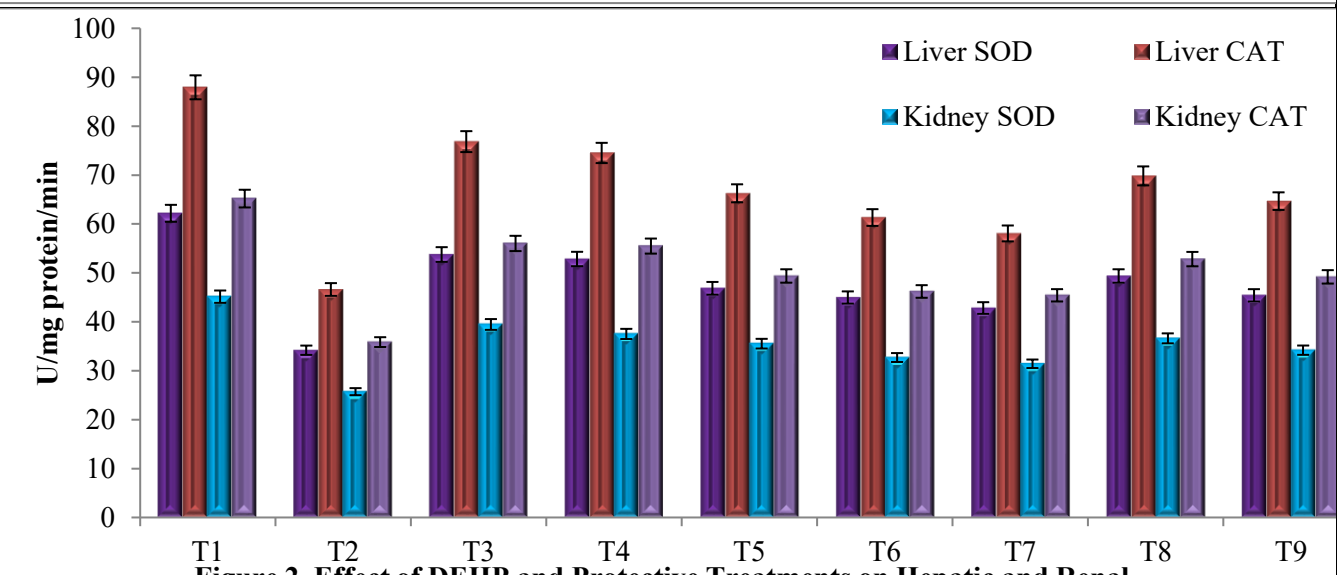

Figure 2. Effect of DEHP and Protective Treatments on Hepatic and Renal Antioxidant Defense System

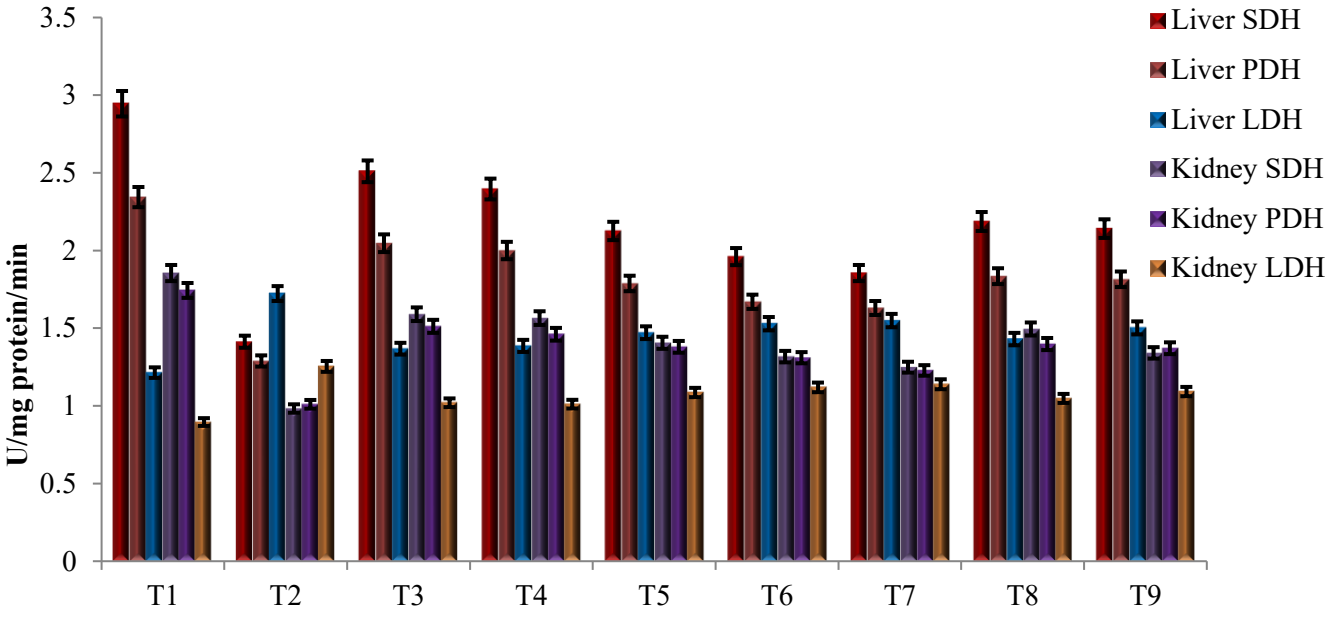

Figure 3. Effect of DEHP and Protective Treatments on Hepatic and Renal…

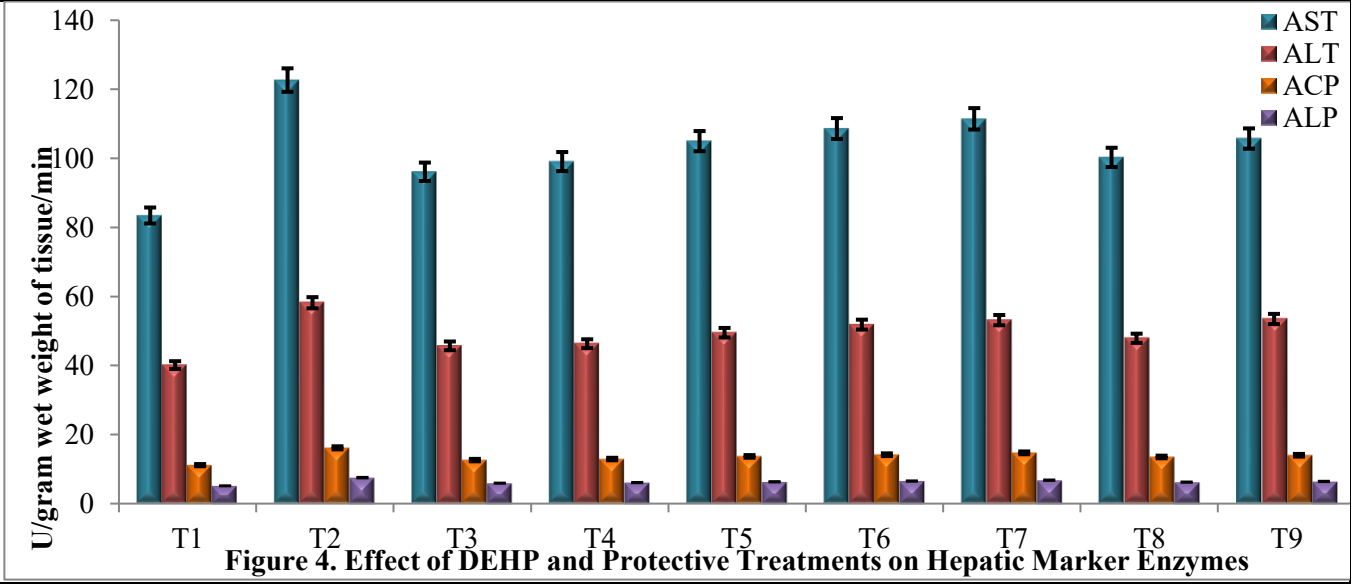

Figure 4. Effect of DEHP and Protective Treatments on Hepatic Marker Enzymes

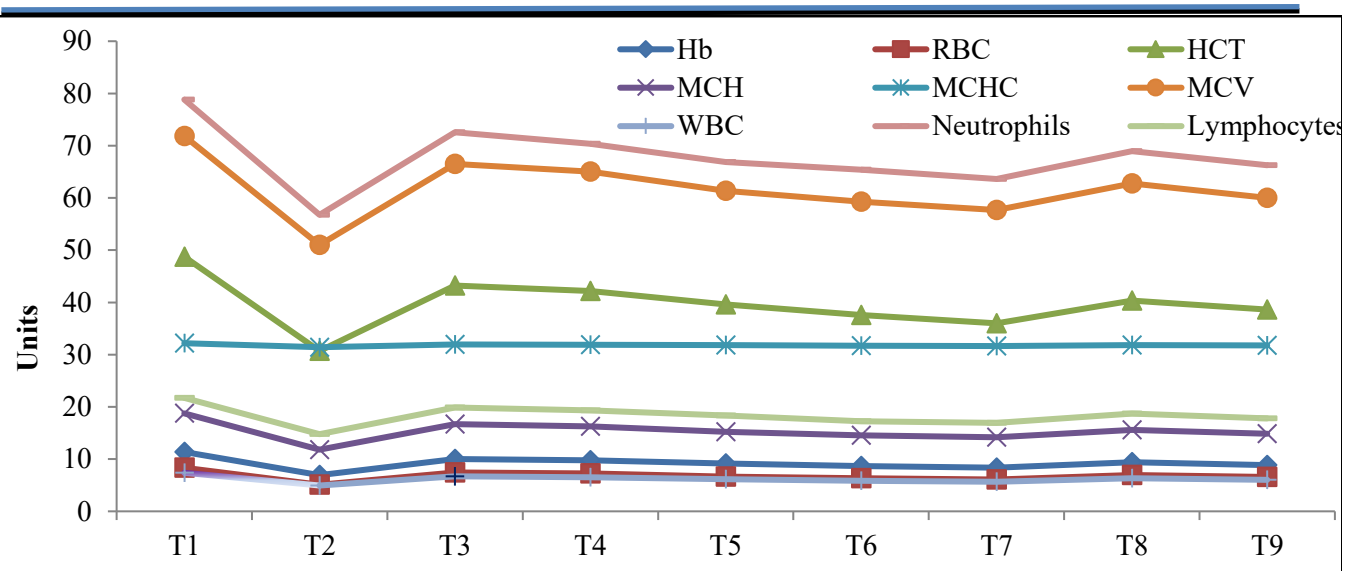

Figure 5. Effect of DEHP and Various Protective Interventions on Hematological Indices

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