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Research Article

THE TOXIC EFFECT OF DI- (2-ETHYLHEXYL) PHTHALATE ON TESTES ANTIOXIDANT AND ENZYME ACTIVITIES IN ADULT MALE RABBITS

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Abstract

Di-2-ethylhexyl phthalate (DEHP) is broadly utilized as a plasticizer in numerous items, particularly therapeutic gadgets, furniture materials, beauty care products, and individual care items. DEHP is non covalently bound to plastics, and so, it'll filter out of these items after rehashed utilize, warming, and/or cleaning of the items. Due to the manhandle of DEHP in various things, it enters and sullies the environment through release from mechanical settings and plastic waste exchange goals. DEHP can enter the body through inward breath, ingestion, and dermal contact on a day by day premise, which has raised a few concerns around its security and its potential impacts on human wellbeing. The purpose of this study was to investigate Di-2-ethylhexyl phthalate (DEHP) to induce testes antioxidant and enzyme activities in adult male rabbits following oral exposure (3 months). Ten male New Zealand white rabbit randomly into two groups: (1): control group and (2): Rabbits were treated daily with di-(2-ethylhexyl) phthalate (DEHP) by gavage at a dose of 500 mg/kg B.W/day (1/50 of DEHP lethal dose. Treatment with DEHP resulted in significant ($P<0.05$) increase in the activities LDH, of testes homogenates AST ALT and TBARS, while ALP, AcP, GSH, GPx, GST, SOD and CAT in testes homogenates was significantly ($P<0.05$) decreased compared with control group.

Keywords: Di-2-ethylhexyl phthalate, Antioxidant, enzyme activities,New-Zealand white rabbits.

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INTRODUCTION

Around 95% of di-ethylhexylphthalate(DEHP) created is utilized as a plasticizer in polyvinyl chloride (PVC) gums for manufacturing adaptable vinyl products[1]. Items ordinarily contain from 1% to 40% DEHP; however,[2] detailed that DEHP levels in PVC therapeutic tubing may be as tall as 80%. Plasticized PVC has been utilized in numerous shopper things and building items, such as tablecloths, shower window ornaments, furniture and vehicle upholstery, impersonation calfskin, plant hoses, floor tiles, swimming-pool liners, sheathing for wire and cable, rainwear, shoes, toys, dolls, infant pants, nourishment bundling materials, tubing utilized in commercial draining hardware, and climate stripping[3]. DEHP is additionally utilized in therapeutic gadgets (blood and intravenous arrangement sacks, catheters, tubing for dialysis and parenteral arrangements, oxygen covers, and pee and colostomy sacks) and in expendable surgical gloves. It has been utilized as a plasticizer in non-PVC materials, counting polyvinyl butyral, characteristic and engineered elastic, chlorinated elastic, ethyl cellulose, and nitrocellulose. In 2005, the breakdown of U.S. utilization of DEHP as a plasticizer was detailed to be 40% for therapeutic gadgets, 30% for shopper products, and 30% for construction-related applications[3]. Non-plasticizer employments of DEHP incorporate its utilize in dielectric liquids for electric capacitors, as an acaricide in plantations, as an idle fixing in pesticides, in restorative items, as a vacuum-pump oil, to identify spills in respirators, and in testing air-filtration frameworks. Be that as it may, a few of these applications are accepted to be now not in utilize or were never carried out on a commercial scale In any case; the utilize of DEHP in a few items has lessened since of wellbeing concerns and administrative restrictions on its utilize. Moreover, DEHP is being supplanted by direct phthalates and other plastomers in numerous other applications, since of their prevalent execution and moo toxicity[1]. Oxidative stress is defined as an impaired balance between free radical production and antioxidant capacity resulting in excess oxidative products. Oxidative stress is reported to play an important role in many pathological conditions including insulin resistance, but it is unknown whether current levels of environmental chemicals in urban areas play a role in the development of the chronic diseases in the adult population[4]. The cells have different mechanisms to alleviate oxidative stress and repair damaged macromolecules. The primary defense is offered by enzymatic and non-enzymatic antioxidants which have been shown to scavenge free radicals and reactive oxygen species (ROS) [5]. Data on phthalates have shown that phthalates were able to produce free radicals by several pathways in germ cells suggesting the possibility that oxidative stress and mitochondrial dysfunction in germ cells may contribute to phthalate-induced disruption of spermatogenesis [6]. DEHP treatment was reported to provoke oxidative stress as measured by increases in ROS in subsequently isolated rat spermatocytes [7]**.** MEHP was reported to increase peroxiredoxin-3 and cyclooxygenase-2 levels in germ cells indicating that the disruption of cellular redox mechanisms occurred in spermatocytes [8]**.** Thus, at least one of the mechanisms underlying the reproductive toxicity of DEHP might be the induction of intracellular ROS and/or to cause alterations on intracellular enzymatic and non-enzymatic antioxidants, thereby producing oxidative stress. Animals treated with phthalates undergo large increases in the activity of H2O2-producing peroxisomal B-oxidation enzymes [9]**,** while causing a minimal induction of H2O2-degrading catalase[10]**.** Consequently, it was hypothesized that an imbalance between H2O2 production and its degradation could lead to H2O2-mediatd oxidative damage, which eventually causes carcinogenesis in the livers of treated rodents [11]**.** [12] investigated the effect of diethyl phthalate, (DEP) on rat testicular antioxidant system. DEP was given in diet to male albino rats at 0.57, 1.43, 2.58 mg/kg diet/day for 150 days. Results showed significant reduction in SOD activities which correlates with a marked increase in lipid peroxidation in a dose dependent manner resulting in impairment of the first line of defense in anti-oxidative reactions. The second line of self-defense against ROS is provided by glutathione peroxidase (GPx) and glutathione reductase (GR) which brings about detoxification of various peroxides and maintains the GSH/GSSG ratio that inhibits lipid peroxidation (LPO) effect on sperm membrane[13]. Decreased levels of both GPx and GR observed in this study, indicates impairment in glutathione metabolism leading to depletion in the GSH/GSSG ratio which could be fatal to the spermatozoa. In a study by[14]**,** administration of DBP at 2.0 g/kg for 9 days to male Wister rats caused significant reduction in the activities of SOD and CAT in the post-mitochondrial fraction of rat

testes accompanied by a significant reduction in testicular GSH status, while the high level of testicular MDA in the DBP-treated rats corresponds to the observation that DBP induces testicular damage through free radical-mediated detrimental effects in testicular tissues.

MATERIALS AND METHODS

In this study di-(2-ethylhexyl) phthalate (DEHP) was used. di- (2-ethylhexyl) phthalate (purity 99.0%) was purchased from Sigma–Aldrich (USA). The chemicals used in the experiment were of analytical grade. Develop male Modern Zealand White rabbits (age of 7 months and beginning weight of (2.917 ± 28.9 gm) were utilized. Animals were individually housed in cages and weighed weekly throughout 3-months experimental period. Ten mature male rabbits were randomly divided into couple equal groups (each five rabbits): Group I**:**abbits were used as control and received an equivalent volume of the vehicle (corn oil) alone by verbal gavage day by day for 12 progressive weeks.. Group II: Rabbits were treated daily with di-(2-ethylhexyl) phthalate (DEHP) by gavage at a dose of 500 mg/kg B.W/day (1/50 of DEHP lethal dose[15,16] **.**Semen collection was done weekly and continued throughout the 12-week experimental period, so 60 ejaculates obtained per treatment. Seminal plasma was obtained by centrifugation of semen samples at 860 ×g for 20 min at (4°C), and was stored at (−20°C) until analysis. The exercises of seminal plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were tested by the strategy of[17]**.** Alkaline phosphatase (AlP; EC 3.1.3.1) activity was determined in plasma according to the method of [20]. Testes were frozen at -20ºC, homogenized and assayed for Lactate dehydrogenase (LDH EC 1.1.1.27) activity was determined by the method of[18]**.** Glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to[19]**.** Catalase (CAT; EC 1.11.1.6) activity was determined using the Luck method involving the decomposition of hydrogen peroxide[20]**.** Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to [21]**.** Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the method of [22]**.** Statistical analysis where applicable, statistical analysis was carried out in Minitab software; statistical significance was assessed using one way ANOVA analysis. After discovery ordinary dissemination to the information and suitable $P < 0.05$ consider note worthy.

RESULTS

Table 1. showed the overall means of the activities of lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AlP) and acid phosphatase (AcP) in seminal plasma as affected by treatment with di-ethylhexylphthalate throughout the 12-week experimental period. Represent the biweekly mean values of these parameters expressed as absolute values. Treatment with DEHP resulted in significant ($P<0.05$) increase in the activities of seminal plasma LDH, AST and ALT, while ALP and AcP was significantly ($P<0.05$) decreased compared with control group. **Table 2.** showed the mean values of the activities of lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AlP) and acid phosphatase (AcP) in testes homogenates as affected by treatment with di-ethylhexylphthalate**.** Treatment with DEHP resulted in significant ($P<0.05$) increase in the activities of testes homogenates LDH, AST and ALT, while ALP and AcP was significantly ($P<0.05$) decreased compared with control group. **Table 3.** The effects of di-ethylhexylphthalate (DEHP) on testes homogenates glutathione (GSH), glutathione peroxidase (GPx), glutathione S- transferase (GST), catalase (CAT) and superoxide dismutase (SOD) activities period are shown in Table 3. Treatment with DEHP caused significant ($P<0.05$) decrease in the activity of GSH, GPx, GST, SOD, CAT and TBARS in testes homogenates.

Table 1: Average of seminal plasma lactate dehydrogenase (LDH; U/L), aspartate transaminase (AST; U/L), alanine transaminase (ALT; U/L), alkaline phosphatase (AlP; U/L) and acid phosphatase (AcP; U/L) of male rabbits treated with di-ethylhexylphthalate (DEHP)

Animal Groups	Lactate dehydrogenase (LDH; U/L)	Aspartate transaminase (AST; U/L)	Alanine transaminase (ALT; U/L)	Alkaline phosphatase (AlP; U/L)	Acid phosphatase (AcP; U/L)
Control (Mean±SE)	1268 ± 14.0^{c}	36.4± 0.22^{b}	25.0± 0.14^{b}	61.7± 0.50^{c}	37.9± 0.15^{b}
DEHP (Mean±SE)	1453 ± 20.7^{a}	39.1 ± 0.23^{a}	27.2± 0.27^{a}	55.6 ± 0.79^{d}	33.3 ± 0.53^{d}

Values are means ± SEM of 5 rabbits in each group. Mean with different letters **(a- d)** are significantly difference ($p \leq 0.05$) at same raw. Mean with the same letters (a-d) are non-significantly difference ($p \geq 0.05$). Table (2). Average of testes homogenates lactate dehydrogenase (LDH; IU/gT), aspartate transaminase (AST; IU/gT), alanine transaminase (ALT; IU/g T), alkaline phosphatase (AlP; IU/g T) and acid phosphates (AcP; IU/g T) in male rabbits treated with di-ethylhexylphthalate (DEHP).

Animal Groups	Lactate dehydrogenase (LDH; IU/gT)	Aspartate transaminase (AST; IU/gT)	Alanine transaminase (ALT; IU/gT)	Alkaline phosphatase (AlP; IU/gT)	Acid phosphatase (AcP; IU/gT)
Control (Mean±SE)	548.3±4.03^{b}	66.6±0.99^{b}	48.8±1.66^{c}	92.3±1.66^{b}	53.6±1.53^{b}
DEHP (Mean±SE)	916.1±5.8^{a}	81.7±0.66^{a}	66.3±0.50^{a}	74.6±1.03^{c}	40.3±1.64^{c}

Values are means ± SEM of 5 rabbits in each group. Mean with different letters (a- d) are significantly difference ($p \leq 0.05$) at same raw. Mean with the same letters (a-d) are non-significantly difference ($p \geq 0.05$). Table (3). Average of testes homogenates glutathione *(*GSH*;* mM/gT), glutathione peroxidase (GPx*;* U/mgT), glutathione S transferase (GST; nmol/min/gT), catalase (CAT*;* nmol/min/gT), superoxide dismutase (SOD*;* U/mgT) and thiobarbituric acid-reactive substances (TBARS; nmol/gT) in male rabbits treated di-ethylhexylphthalate (DEHP).

Animal Groups	Glutathione (GSH; mM/gT)	Glutathione peroxidase (GPx; U/mgT)	Glutathione S-transferase (GST; nmol/min/gT)	Catalase (CAT; nmol/min/g T)	Superoxide dismutase (SOD; U/mgT)	Thiobarbituric acid-reactive substances (TBARS; nmol/gT)
Control (Mean± SE)	4.48±0.99^{b}	13.54±0.99^{b}	0.123±0.99^{b}	5.22±0.99^{b}	12.28±0.99^{b}	60.05±0.99^{b}
DEHP (Mean± SE)	3.26±0.8^{c}	6.86±0.8^{d}	0.052±0.8^{c}	3.94±0.8^{c}	9.02±0.8^{c}	40.54±0.52^{c}

Values are means ± SEM of 5 rabbits in each group. Mean with different letters (a- d) are significantly difference ($p \leq 0.05$) at same raw. Mean with the same letters (a-d) are non-significantly difference ($p \geq 0.05$).

DISCUSSION

The prepubertal testis has classically been characterized as a tranquil organ, but later discoveries uncovered that prepuberty may be a basic window for male reproductive framework improvement; amid this or ganize the method of testicular spermatogenesis and steroidogenesis is profoundly responsive to EDCs [23], finally resulting in disturbed spermatogenesis and higher incidence of testicular germ cell cancer

[24]. Our comes about appeared presentation to DEHP caused disability of testicular antioxidative protein exercises, variation of the proportion of GSH. The testicular antioxidative enzymes activities were closely related to the dose of DEHP, which showed the trend of decline as the dose increases All those results shown that prepubertal presentation to tall measurements of DEHP seem essentially delay tubule advancement of the testis amid adolescence and have long-term impact on grown-up spermatogenesis It has been affirmed that controlled and moo levels of oxidative push are fundamental for ordinary testicul ar work, which were produced by two imperative, tall energy-demanding capacities, spermatogenesis and steroidogenesis.In normal physiological state, testes are equipped with potent antioxidant system that protects it against ROS damage[25]. The present study showed that DEHP caused changes in the activities of marker enzymes like ALT, AST, AlP and AcP in seminal plasma and testes (Tables 1). The decrease in seminal plasma enzyme activities in diethylhexylphthalate-exposed rabbits may be due to altered function of male accessory sex glands [26]. [27] reported that increased serum alanine transaminase (ALT) and aspartate transaminase (AST) activities are indicator on hepatocellular injury with membrane damage or necrosis of liver cells. Similarly, phosphatases are enzymes that catalyze the splitting of phosphoric acids from certain monophosphoric esters, a reaction of considerable importance in several body processes. Alkaline phosphatase (AlP) and acid phosphatase (AcP) have been directly implicated in the extent of cellular damage and toxicity, particularly of liver and cardiac tissue. The primary importance of measuring AlP is to check the possibility of mainly liver or bone diseases. The increase in the activity of enzymes like ALT, AST, AlP and AcP in plasma could reflect the state of hepatotoxicity. Also,[28] suggested that the increase in the activities of AlP and AcP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis. [29] reported that the decrease of AST and ALT activity in tissues may be interpreted as a compromise of the tissues integrity. In spite of the fact that, ALT and AST are "marker" chemicals for the liver.. It is believed that any alteration at the subcellular level may affect the activity of these enzymes in other tissues[30]. Acid phosphatases act as marker enzymes for the detection of lysosomes in cell fractions and can be altered by the presence of xenobiotics[31], whilst alkaline phosphatases are intrinsic plasma membrane enzymes found on the membranes of almost all animal cells. Both enzymatic activities have been studied in several organisms and the influence of heavy metals has been reported[32]. These enzymatic activities are involved in a variety of metabolic processes, such as molecule permeability, growth and cell differentiation and steroidgenesis[33]. Activity of enzymes assayed in the liver of the experimental animals is consistent with the observation on serum protein concentrations. ALP catalyses the hydrolysis of organic phosphates at alkaline pH. ALP activity gives an indication of possibility of liver diseases[34]. ACP catalyses the removal of phosphoryl group from a phosphate ester in an acidic medium. It is found throughout the body[34]. [35] suggested that the activities of lactate dehydrogenase (LDH) were significantly increased at doses from 500 mg/kg bw of DEHP. The creators recommended that DEHP can influence spermatogenesis in grown-up rats by modifying the exercises of LDH mindful for the development of perms which the decreased number ofsperms may be responsible for the anti-fertile effects of DEHP. Glutathione S-transferase plays a key role in cellular detoxification by catalyzing the reaction of glutathione with toxicants to form an S-substituted glutathione[36]. Superoxide dismutase has an antitoxic effect against the superoxide anion; SOD accelerates the dismutation of superoxide to H2O2 which is removed by catalase[37]. Thus SOD can be acting as a primary defense and prevents further generation of free radicals. While, catalase catalyzes the removal of H2O2 that formed during the reaction by SOD [38].

The present study showed that DEHP caused decreased in the activity and concentration of antioxidant enzyme GSH, GPx, GST, SOD and CAT in testes (Tables 3). The decrease in antioxidant enzyme and GSH corroborated the findings of[39] who found a decrease in the activities of testicular antioxidant enzymes in mice. Also, those authors reported ROS-induced impairment of Leydig cells, which play a pivotal role in steroidogenesis leading to reduced synthesis of testosterone. Thus, the observed increase in free radicals could be attributed in part to the concomitant reduction of antioxidant enzyme and GSH activity following DEHP treatment.

CONCLUSION

It is clear from the obtained results that diethylhexylphthalate induced pronounced hazardous effects in testes antioxidant and enzyme activities. This effect may decrease the productive and reproductive performance of animals. Also, the measured parameters could be used as bioindicators for the negative effect and reproductive toxicity of the exposure to diethylhexylphthalate . Attention of safety precautions should be taken during usage and exposure to diethylhexylphthalate to avoid its harmful effect.

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