Effect of some antioxidants on experimental testicular injury induced in rats

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Abstract

The present study aimed to evaluate the protective role of resveratrol and curcumin on oxidative testicular damage induced by di-(2-ethylhexyl) phthalate (DEHP). Male Wistar rats were divided into six groups; three groups received oral daily doses of DEHP (2 g/kg BW) for 45 days to induce testicular injury. Two of these groups received either resveratrol (80 mg/kg BW) or curcumin (200 mg/kg BW) orally for 30 days before and 45 days after DEHP administration. A vehicle-treated control group was also included. Another two groups of rats received either resveratrol or curcumin alone. Oxidative damage was observed by decreased levels of total antioxidant capacity (TAC) and glutathione (GSH) and increased malondialdehyde (MDA) level in the testes of DEHP-administered rats. Serum testosterone level as well as testicular marker enzymes activities; acid and alkaline phosphatases (ACP and ALP) and lactate dehydrogenase (LDH) showed severe declines. DEHP administration caused significant increases in the testicular gene expression levels of Nrf2, HO-1, HSP60, HSP70 and HSP90 as well as a significant decrease in c-Kit when compared with the control group. Histopathological observations provided evidence for the biochemical and molecular analysis. These DEHP-induced pathological alterations were attenuated by pretreatment with resveratrol and curcumin. We conclude that DEHP-induced injuries in biochemical, molecular and histological structure of testis were recovered by pretreatment with resveratrol and curcumin. The chemoprotective effects of these compounds may be due to their intrinsic antioxidant properties along with boosting Nrf2, HSP 60, HSP 70 and HSP 90 gene expression levels and as such may be useful potential tools in combating DEHP-induced testicular dysfunction.

Keywords: DEHP; Nrf2; HO-1; HSPs; Resveratrol; Curcumin; Testes; Rat

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Aim of the work

There is increasing public concern that environmental toxicants have the potential to impair human fertility (Colborn et al., 1993). The testis is sensitive to a variety of stressors, such as hyperthermia, inflammation, radiation and exposure to agents that induce apoptosis of germ cells (Richburg, 2000).

Exposure to phthalic acid esters is one of the most common causes of testicular injury. Phthalic acid esters are widely used as plasticizers in several plastic formulations such as polyvinyl chloride (PVC). Among various phthalate esters, di-(2-ethylhexyl) phthalate (DEHP) is the most widely studied toxicants of the male reproductive organs (Harris et al., 1997; Ohashi et al., 2005). DEHP contamination is widespread as PVC containing DEHP is widely used in the manufacture of consumer goods, food containers, toys and medical instruments. The general population is exposed to DEHP through food, water and air, which raises concerns about its hazardous effect on human reproductive organs (Hoyer, 2001).

Administration of DEHP was found to reduce the fertility and induces testicular injury of laboratory animals (Thomas and Thomas, 1984; Oishi, 1986). After oral exposure, most DEHP is rapidly metabolized in the gut into mono-(2-ethylhexyl) phthalate (MEHP), the active metabolite which induces testicular injury (Pollack et al., 1985; Gray and Gangolli, 1986) through disruption of the functions of Sertoli cell and Leydig cell (Lee et al., 1999; Richburg et al., 2002). Furthermore, MEHP induces oxidative stress in germ cells and causes apoptosis of spermatocytes as a direct action of MEHP on the germ cells (Kasahara et al., 2002). It was shown that spermatogenic disturbance induced by DEHP can be prevented by treatment with antioxidants (Ishihara et al., 2000).
Nuclear factor E2-related factor-2 (Nrf2) is a transcription factor, which plays a key role in the cellular defense against oxidative and xenobiotic stresses through its capability to induce the expression of genes, which encode detoxifying enzymes such as heme oxygenase-1 (HO-1) and antioxidant proteins (Jaiswal, 2004).

Heat-shock proteins (HSPs), or stress proteins, are a class of functionally related highly conserved proteins, found in virtually all living organisms and are named according to their molecular weight (Li and Srivastava, 2004). HSP60, HSP70 and HSP90 are also known as chaperones, play crucial roles in folding/unfolding of proteins, assembly of multiprotein complexes, transport/sorting of proteins into correct subcellular compartments, cell-cycle control and signaling, and protection of cells against stress/apoptosis (Sōti et al., 2005).

Resveratrol (3, 4', 5-trihydroxy-trans-stilbene) is a natural polyphenol obtained from grapes, mulberries, red wines and root extracts of the weed Polygonum Cuspidatum. It is involved in numerous cellular responses including cell cycle arrest, apoptosis and differentiation, and has anti-inflammatory, anticancer, antioxidant properties (Brisdelli et al., 2009). It also exhibits a protective effect against lipoperoxidation in cell membranes and against the DNA damage caused by reactive oxygen species (ROS) (Revel et al., 2001). Resveratrol has been demonstrated to protect sperm from apoptosis (Revel et al., 2001) and increase sperm output (Juan et al., 2005).

Curcumin [(1, 7- bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione)], is a major active component of the turmeric, which is obtained from Curcuma longa rhizomes (Zengiberaceae family). It has been widely used as a spice to give the specific flavour and yellow colour to curry in
many countries in Asia and Africa (Joe et al., 2004). Curcumin has been shown to be a powerful inhibitor of the proliferation of several tumour cells. It exhibits anticlastogenic, antiviral, anti-infective and antioxidant properties (Araujo and Leon, 2001). Curcumin was demonstrated to prevent peroxidative changes in the sperm and the testicular membrane, thus enhancing sperm motility and decreasing spermatozoa abnormalities (Farombi et al., 2007).

Therefore, the present study aimed to evaluate the protective effects of resveratrol and curcumin against DEHP-induced testicular injury in rats.
Introduction

The testes

The testes are paired symmetrical organs located within the scrotum (Mihmanli and Kantarci, 2009). The testes are mainly comprised of tightly coiled seminiferous tubules, which are supported by loose interstitial connective tissue (Griffin and Wilson, 1998). Microscopically, the wall of the seminiferous tubules consists of a lamina propria and a multilayered germinal epithelium. In the midst of the germinal epithelium, sperm cells and supporting Sertoli cells can be identified. Leydig interstitial cells, which have an endocrine function, are found in the connective tissue between the seminiferous tubules as shown in Fig. 1. (Kandeel et al., 2001).

![Microscopical structure of the seminiferous tubule](image)

Fig. 1. Microscopical structure of the seminiferous tubule (Wagner et al., 2008).

The Sertoli cells play a major role in regulation of spermatogenesis and altering rates of the produced spermatozoa. Sertoli cell functions include providing structural support and nutrition to the developing germ cells, phagocytosis of degenerating germ cells, release of spermatids at spermiation and production of proteins that regulate and/or respond to pituitary hormone release and that influence mitotic activity of spermatogonia (Russell and Griswold, 2000). Also, the functions involve control of the blood testis...
barrier, production of tubule fluid, paracrine control of Leydig cells and Peritubular cells (Gupta, 2005).

Sertoli cells produce androgen binding proteins, inhibin (Griswold, 1988), polyamines (Tsai and Lin, 1985), growth factors (Feig et al., 1983), lactate and pyruvate (Jutte et al., 1983) and other α-keto acids (α-ketoisovalerate, α-ketoisocaproate, etc.) (Williams and Foster, 1988). It is being noted that lactate, pyruvate and other α-keto acids are critical for maintaining germ cells. Isolated spermatocytes prefer utilizing α-keto acids rather than glucose as an energy source. Secreted α-keto acids may be used via the germ cell specific lactate dehydrogenase-X (LDH-X) isozyme, which converts α-keto acids to α-hydroxyacids, thus shuttling reducing equivalents into germ cell mitochondria (Alves et al., 2013).

Leydig cells are the major cell type within the interstitium, found adjacent to blood vessels and seminiferous tubules (Schulze, 1983). They are the primary source of testicular steroids, including testosterone, progesterone and probably estrogen under the effect of luteinizing hormone (LH) (Tremblay, 2015).

Peritubular cells, together with collagen and other extracellular matrix components in the boundary tissue, form the tubular wall. Oxytocin, secreted by leydig cells, acts upon the peritubular cells to cause their contraction, which propels the released sperm towards the rete testis (Heindel and Treinen, 1989). The peritubular cells secrete factors referred to “P-mod-S” (Peritubular modifier of Sertoli cells), which has been suggested to be stimulated by Leydig cell androgens, which then modulates specific Sertoli cells functions (Skinner et al., 1988). This type of paracrine communication between the three types of cells exemplifies the complex interactions necessary for proper testis regulation.
Environmental toxicants and male reproductive functions

There is a wide variety of environmental toxicants. Endocrine disruptors are probably the best-known environmental toxicants because they disrupt the endocrine system, which regulates growth, development, sexual maturity and brain function in organisms (Benson et al., 2001). Numbers of endocrine-disrupting compounds are found in the environment as a result of industrial and manufacturing activities. These compounds include phthalates, bisphenol A, polychlorinated biphenyls, pesticides, polybrominated diphenyl ethers and others (e.g., cadmium chloride) (Meeker, 2010). These environmental toxicants produce their effects by perturbating the reproductive system either by direct or indirect interaction with vital cellular components or processes and disruption of endocrine relations essential for reproduction (Mattison and Thomford, 1989). This may also be due to induction of reactive oxygen species (ROS) and its associated oxidative stress (Saradha and Mathur, 2006).

Following exposure to a testicular toxicant, the compound must be distributed to the target organs (gonad, hypothalamus, pituitary, epididymis, etc.) where it exerts its toxic adverse effect by interacting with a critical cell or subcellular components. This toxic effect may be very specific, affecting only a single function of a single cell type, or broad and non specific with multiple sites of toxicity within the organism (Mattison and Thomford, 1989). Moreover, the direct-acting testicular toxicants are either chemically reactive or agonists of endogenous molecules. On the other hand, the indirect-acting testicular toxicants are either metabolized to direct-acting toxicants or disrupt the endocrine system needed for successful function (Mattison et al., 1990).
Phthalates

General chemical structure of phthalates (1,2-benzenedicarboxylic acid). \(R\) and \(R' = \text{CnH}_{2n+1}\) where \(n = 1-13\)

Phthalates or phthalic acid esters are composed of paired ester groups on a benzene ring. They are synthetic compounds, which have been used in industry since the 1930s. Phthalates are colorless, odorless liquids produced by reacting phthalic anhydride with an appropriate straight or branched chain alcohol that range from methanol and ethanol (C1/C2) up to tridecyl alcohol (C13). Phthalates are mainly used as plasticizers (substances added to plastics to increase their flexibility, transparency, durability, and longevity) and also as additives in a number of various products: insecticides, paints, packagings, cosmetics, coverings, clothes or insulators in electric disposals. Therefore, many consumer products contain specific members of this family of chemicals, including building materials, household furnishings, pharmaceuticals, nutritional supplements, medical devices, dentures, children's toys, glow sticks, modeling clay, food packaging, automobiles, lubricants, waxes and cleaning materials (Heudorf et al., 2007) (Table 1). Approximately seven million tonnes of plasticizers are consumed globally every year. They contribute 10-60% of plasticized products by weight (Rudel and Perovich, 2009)
Di-(2-ethylhexyl) phthalate

Di-(2-ethylhexyl) phthalate is the most widely used plasticizer in manufacturing of articles made of PVC (Heudorf et al., 2007). Di-(2-ethylhexyl) phthalate is an organic compound with the formula C₆H₄(C₈H₁₇COO)₂, being the diester of phthalic acid and the branched chain 2-ethylhexanol. Plastics may contain 1% to 40% of DEHP. Because of its low cost, extensive use and evidence for endocrine disruption, DEHP has received more regulatory and scientific attention than other phthalates (Heudorf et al., 2007).

![Chemical structure of DEHP](Howard, 1996).

Di-(2-ethylhexyl) phthalate is slightly soluble in water and carbon tetrachloride, miscible with mineral oil and hexane, and soluble in blood and body fluids containing lipoproteins. It has many synonyms such as Bis-(2-ethylhexyl) phthalate (BEHP) and dioctyl phthalate (DOP) (Heudorf et al., 2007).

Ingestion, inhalation, intravenous injection and skin absorption are potential pathways of exposure (Schettler, 2006). Human exposure to phthalates can occur as a result of direct contact or use of a product containing phthalates, through the leaching of phthalates as may occur with food packaging, intravenous fluids or by general contamination of the ambient environment (Aurela et al., 1999).
The typical human exposure to DEHP ranges from 3 to 30 µg/kg/day (Doull et al., 1999) but, can be exceeded in specific medical conditions reaching 1.5 mg/kg/day exposure in hemodialysis patients, or as high as 10-20 mg/kg day during neonatal transfusion or parenteral nutrition (Loff et al., 2000; Kavlock et al., 2006). Indeed, critically ill patients and neonates hospitalized in intensive care units may be exposed to significantly higher doses of phthalates that migrate from medical devices such as blood bags, catheters and nasogastric and intravenous tubes (Koch et al., 2006).

Phthalate esters are easily absorbed from the skin or lungs because of their lipophilic nature. Phthalate esters, principally DEHP, can also be introduced directly into the circulatory system by the use of plasticized PVC medical equipment (e.g. tubing) or by infusions from plasticized PVC blood bags. For other than occupationally or medically exposed populations, however, the most common mode of human contact with phthalate esters is ingestion with food or liquids (Kluwe, 1982).

Virtually, all DEHP is protein bound, approximately 80% to lipoproteins and the rest to albumin. Albro and Corbett (1978) examined tissue accumulation of DEHP in rats by monitoring radioactivity in fat and liver tissue after dietary exposure to $^{14}$C-DEHP. Steady-state concentrations in liver were achieved within a week of initiation of treatment, while that in fat required two weeks.

Fat absorptive organs (gastrointestinal tract) and excretory organs (liver, kidney and gastrointestinal tract) are the major initial repositories for the dialkyl esters (Kluwe, 1982). After exposure, DEHP is rapidly hydrolyzed by esterases in the gut, liver and blood into mono-(2-ethylhexyl) phthalate (MEHP), which is believed to be the active molecule (Gray and Gangolli, 1986; Koch et al., 2005).
The major route of phthalate ester elimination in both rodent and man is urinary excretion. Appreciable percentages of DEHP appeared in feces when the compound was given by gavage (Kluwe, 1982). Di-(2-ethylhexyl) phthalate can also undergo biliary excretion, as indicated by the report of Daniel and Bratt (1974), where 14% of an oral dose of 2.6 mg/kg was recovered from bile. Extraction of 28% of an intravenous dose of 50 mg/kg DEHP from feces further indicates that biliary excretion can be a significant route of phthalate ester elimination.

Exposure to DEHP produced hepatocellular carcinoma in rodents along with a variety of other hepatocellular effects, such as proliferation of peroxisomes and mitochondria, liver tissue proliferation and suppression of apoptosis (Heudorf et al., 2007). Di-(2-ethylhexyl) phthalate has been shown to cause renal cysts in rodents and also produce renal peroxisome proliferation. Since haemodialysis is a route of exposure to DEHP, renal cysts have been noted in hemodialysis patients (Woodward, 1990).

In animal studies, rats with diets contaminated with DEHP were found to have thyroid alterations and lower plasma thyroxin concentrations compared with controls (Howarth et al., 2001). In addition, on in-vitro study reported that DEHP and other phthalates caused changes in the iodide uptake of thyroid follicular cells (Wenzel et al., 2005).

In adult rats, respiratory distress and dose-dependent lethality occur after a single intravenous injection of 200-300 mg/kg DEHP (Schulz et al., 1975), while a 4-week-long aerosol treatment (estimated dose 230 mg/kg/day) causes relative lung weight increase, accompanied by foam-cell proliferation and alveolar septa thickening (Klimisch et al., 1992). Di-(2-ethylhexyl) phthalate induced release of lysosomal enzymes from cultured alveolar macrophages, as well as constriction and oedema of pulmonary vessels in isolated perfused rat heart-lung preparations (Labow et al., 1990).
In animal models, experimental data have established phthalate toxicity on testicular function in prenatal, neonatal, and postnatal rats. Pregnant rats exposed to DEHP gave birth to fetuses with reproductive tract defects such as hypospadias, decreased anogenital distance, retained nipples, malformed seminal vesicles, and cryptorchidism (Jiang et al., 2007; Rider et al., 2008). Phthalate exposure during pregnancy results in adverse effects on the male rat reproductive tract that are indicative of a suppression of the androgen pathway, thus phthalates are classified as anti-androgenic chemicals (Earl Gray et al., 2006).

Adverse effects of DEHP on testes were observed after administration of approximately 3mg/kg bodyweight to pregnant and lactating rats (Arcadi et al., 1998) while adult rats were affected after administration of approximately 1100mg/kg (Agarwal et al., 1986). In addition, in studies where effects on the testes have been compared in rats of different ages, the younger rats have proven more sensitive, indicating age-toxicokinetic relationship (Sjöberg et al., 1986).

Sertoli cells have been cited as the primary cellular targets of testicular MEHP induced toxicity (Li and Kim, 2003). Heindel and chapin (1989) determined that the MEHP impairs the ability of FSH to bind to Sertoli cell receptors, reducing cyclic adenosine monophosphate (cAMP) accumulation in Sertoli cells. Subsequent research suggested that MEHP may be acting at the level of the GTP-binding protein, which couples the FSH receptor to the cAMP messenger system. MEHP interference with the GTP-binding protein disrupts the signal transduction pathway, causing decreased cAMP accumulation and impairing Sertoli cell function (Grasso et al., 1993). Although Sertoli cells were thought to be the primary targets of phthalate exposure in testis (Grasso et al., 1993), available data suggest that Leydig cells are also one of the main targets (Ge et al., 2007).
Testicular injury caused by DEHP in animal models is characterized by marked degeneration of seminiferous tubules resulting in testicular atrophy (Gray and Butterworth, 1980; Oishi, 1985). Although many anti-androgenic compounds antagonize the androgen receptor (AR), phthalate esters do not bind the human AR in-vitro at physiological concentrations (Parks et al., 2000); the absence of AR binding has been observed with DEHP and its metabolite MEHP (Parks et al., 2000) as well as DBP and its metabolite mono butyl phthalate (MBP) (Foster et al., 2001). In addition, Corton and Lapinskas (2005) highlighted the participation of peroxisome proliferator-activated receptor (PPAR) subtypes as potential mediators of phthalate-induced effects on the male reproductive tract. Another mechanistic pathway is activation of metabolizing enzymes leading to free radical production and oxidative stress cascade (O'Brien et al., 2005).

**Resveratrol and male reproductive system**

Antioxidant activity of resveratrol has been demonstrated in a variety of animal models of oxidative testicular injury such as benzo (a) pyrene (Revel et al., 2001), ischemia-reperfusion model (Uguralp et al., 2005), ethanol (Kasdallah-Grissa et al., 2006) and acrylamide (Alturfan et al., 2012). Resveratrol has been reported to have protective effects on male reproductive system

**The protective effect of resveratrol on male reproductive system.**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Increase sperm output and protection of sperm from apoptosis caused by physical damage.</td>
<td>(Juan et al., 2005).</td>
</tr>
<tr>
<td>Improvement of sperm development and increase sperm output as well as protection of sperm from DNA damage caused by benzo (a) pyrene.</td>
<td>(Revel et al., 2001).</td>
</tr>
<tr>
<td>Protection of sperm from apoptosis caused by ischemia-reperfusion (I/R) injury in testicular torsion cases.</td>
<td>(Uguralp et al., 2005)</td>
</tr>
</tbody>
</table>
Induction of spermatogenesis and enhancement of expression of c-kit after 2,5-hexanodione induced testicular injury in rats.  
(Jiang et al., 2008).

A stimulatory effect on the secretion of FSH and LH through feedback regulation of hypothalamic-pituitary-gonadal axis as well as acting as an estrogen agonist due to its structural similarity with estrogen, which could elevate the concentration of FSH and testosterone.  
(Juan et al., 2005).

Inhibition of lipid peroxidation induced by chronic ethyl alcohol intake on rat testis.  
(Kasdallah-Grissa et al., 2006).

Protection of rat sperm and testes from changes induced by hyperthyroidism such as loss of sperm motility and oxidative stress.  
(Ourique et al., 2013).

A protective effect against testicular damage induced by DBP demonstrated by the increase in the expression of c-kit, inhibition of apoptosis of Leydig cells and improvement of spermatogenesis along with protection of ductus epididymis and ductus deferens in rats.  
(Ünal et al., 2013; Sahin et al., 2014).

### Curcumin and male reproductive system

Curcumin has been reported to have protective effects on male reproductive system.

**The protective effect of curcumin on male reproductive system.**

<table>
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<tr>
<th>Effect</th>
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<tbody>
<tr>
<td>Ameliorating the decrease in tubule volume, length, diameter and height of germinal epithelium, and also Leydig cell hyperplasia caused by metronidazole (an anti-parasitic drug, acts as a testosterone biosynthesis inhibitor) in the testes of male mice as well as enhancement of serum level of testosterone.</td>
<td>(Noorafshan et al., 2011).</td>
</tr>
<tr>
<td>Restoration of the antioxidant enzymes, G6PD and gamma-glutamyl transferase (γ-GT), enhancement of sperm motility and decrease in spermatozoa defects in rats induced by DBP.</td>
<td>(Farombi et al., 2007).</td>
</tr>
<tr>
<td>Improvement of aflatoxin-induced sperm decrease, immobilization, and viability as well as enhancement of the morphological characteristics of sperm in mice.</td>
<td>(Mathuria and Verma, 2008).</td>
</tr>
<tr>
<td>Protection of Leydig cells of mice from the damage caused by chronic alcohol consumption demonstrated by decreased both necrosis of Leydig cells and diameter of mitochondria.</td>
<td>(Giannessi et al., 2008).</td>
</tr>
<tr>
<td>Protective effects on cisplatin induced irregularity of seminiferous tubules, reduction of seminiferous epithelial layers, sperm maturation arrest, and perivascular fibrosis in rats demonstrated by a major increase in plasma testosterone level, GSH level and GPX activity, and a decrease in MDA and NO levels in testicular tissue.</td>
<td>(Ilbey et al., 2009).</td>
</tr>
<tr>
<td>A potential agent for the prevention of cancer progression, or at least of the initial phase of metastasis, in prostate cancer in both in vitro and in vivo.</td>
<td>(Hong et al., 2006).</td>
</tr>
<tr>
<td>Curcumin analogues had been studied as novel androgen receptor antagonists with the potential to act as an anti-prostate cancer agent as they may function as a 17α-substituted dehydrotestosterone, thus, some of these compounds have been identified as a new class of anti-androgen agents.</td>
<td>(Ohtsu et al., 2002).</td>
</tr>
</tbody>
</table>

**Summary and conclusion**

Exposure to phthalic acid esters is one of the most common causes of testicular injury. Phthalic acid esters are widely used as plasticizers in several plastic formulations such as PVC. Among various phthalate esters, DEHP is the most widely studied toxicants of the male reproductive organs since DEHP is widely used in the manufacture of consumer goods, food containers, toys and medical instruments, DEHP contamination is widespread, and the general population is exposed to DEHP through the food, water and air, which raises concerns about human reproductive hazards.

Administration of DEHP was found to reduce the fertility and induces testicular injury of laboratory animals. After oral exposure, most DEHP is rapidly metabolized in the gut into MEHP, the active metabolite which
induces testicular injury through disruption of the function of Sertoli cell and Leydig cell. Furthermore, MEHP induces oxidative stress in germ cells and causes apoptosis of spermatocytes as a direct action of MEHP on the germ cells. The present study aimed to evaluate the protective effects of resveratrol and curcumin against DEHP-induced testicular injury in rats.

Testicular injury was assessed by measuring the testicular ALP, ACP and LDH activities along with serum testosterone level. In addition, the antioxidant status; TAC, GSH and MDA, testicular gene expression levels of Nrf2, HO-1, HSP60, HSP70, HSP90 and c-Kit, and histopathological examination of testicular tissues were estimated.

Male Wistar rats were divided into six groups. Three groups received oral daily doses of DEHP at a dose of 2g/kg body weight for 45 days. Two of these groups were pretreated orally with either resveratrol or curcumin, daily at the doses of 80 mg/kg body weight and 200 mg/kg body weight, respectively for 30 days prior and 45 days after DEHP administration. A vehicle-treated control group was also included. Two groups of rats received either resveratrol or curcumin only.

DEHP-induced testicular toxicity was manifested by the significant decrease of body weight gain and testes weight. In addition, the significant decrease in GSH and TAC levels and increase in MDA level in the testicular tissues compared to the control group might be an evidence for DEHP-induced oxidative stress. Administration of DEHP resulted in a severe testicular damage revealed by a significant decline in serum testosterone level and the testicular activities of ALP, ACP and LDH. Moreover, the results revealed significant increase in testicular gene expression levels of Nrf2, HO-1, HSP60, HSP70 and HSP90 along with significant decrease in the expression level of testicular c-kit gene in the DEHP group compared with the control group. These results were further confirmed by histopathological
studies as DEHP administration resulted in testicular atrophy, degeneration, impaired spermatogenesis, hyalinization of spermatids in the seminiferous tubules lumen, focal haemorrhage and oedema.

Resveratrol or curcumin administration resulted in a significant increase in the reduced body weight gain, testes weight and testicular TAC level when compared with the DEHP-administered group. In addition, normalization of serum testosterone level, testicular GSH and MDA levels as well as testicular LDH activity was achieved in the DEHP group treated with either resveratrol or curcumin when compared with the control group. Moreover, resveratrol or curcumin administration resulted in a significant increase in the reduced testicular ALP and ACP activities when compared with the DEHP-group.

Results of the present study revealed that resveratrol or curcumin pretreatment resulted in a significant increase in testicular gene expression levels of Nrf2, HO-1, HSP60, HSP70 and HSP90 when compared with the DEHP-group. Resveratrol resulted in normalization of expression level of testicular c-kit while curcumin resulted in a significant increase in testicular gene expression levels of c-kit when compared with the DEHP-treated group. These findings were further confirmed by the histopathological studies where resveratrol and curcumin pretreatment resulted in significant improvement in the testicular tissues of rats. No change in all studied parameters was revealed in the group received resveratrol or curcumin only compared with the control rats, which suggests safety of resveratrol and curcumin dosages used in the present study.

In the present study, such protective effects of resveratrol and curcumin could be attributed mainly to their antioxidant properties. Resveratrol and curcumin have been found to have a wide range of beneficial effects as a natural antioxidant by scavenging free radicals, chelating metal ions along with inhibition of lipid peroxidation. It was also reported that resveratrol has
a stimulatory effect on the secretion of FSH and LH, the major endocrine gonadotrophins regulators of spermatogenesis. In addition, curcumin was shown to induce several enzymatic antioxidants such as catalase and induce de novo synthesis of GSH.

It can be concluded that, DEHP caused testicular injury as indicated by the different biochemical, molecular and histopathological findings of the present study. Lipid peroxidation and oxidative stress are implicated in the pathogenesis of DEHP-induced adverse effects. The testicular injury induced by DEHP seemed to be effectively modulated by the use of resveratrol and curcumin. The beneficial effects of these compounds may be due to their intrinsic antioxidant properties along with boosting Nrf2, HSP60, HSP70 and HSP90 gene expression levels and as such may be useful potential tools in combating DEHP-induced testicular dysfunction.
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(516x228) Abstract (516x173) باللغة العربية: (516x159) ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا ﺻا 

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معدل زيادة الوزن وزن الخصيين وكذلك القدرة الكلية لمضادات الأكسدة وكذلك نشاط خسائر الفوسفاتيز القلوي

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معدل زيادة الوزن وزن الخصيين وكذلك القدرة الكلية لمضادات الأكسدة وكذلك نشاط خسائر الفوسفاتيز القلوي
The present study aimed to evaluate the protective role of resveratrol and curcumin on oxidative testicular damage induced by di-(2-ethylhexyl) phthalate (DEHP). Male Wistar rats were divided into six groups; three groups received oral daily doses of DEHP (2 g/kg BW) for 45 days to induce testicular injury. Two of these groups received either resveratrol (80 mg/kg BW) or curcumin (200 mg/kg BW) orally for 30 days before and 45 days after DEHP administration. A vehicle-treated control group was also included. Another two groups of rats received either resveratrol or curcumin alone. Oxidative damage was observed by decreased levels of total antioxidant capacity (TAC) and glutathione (GSH) and increased malondialdehyde (MDA) level in the testes of DEHP-administered rats. Serum testosterone level as well as testicular marker enzymes activities; acid and alkaline phosphatases (ACP and ALP) and lactate dehydrogenase (LDH) showed severe declines. DEHP administration caused significant increases in the testicular gene expression levels of Nrf2, HO-1, HSP60, HSP70 and HSP90 as well as a significant decrease in c-Kit when compared with the control group. Histopathological observations provided evidence for the biochemical and molecular analysis. These DEHP-induced pathological alterations were attenuated by pretreatment with resveratrol and curcumin. We conclude that DEHP-induced injuries in biochemical, molecular and histological structure of testis were recovered by pretreatment with resveratrol and curcumin. The chemoprotective effects of these compounds may be due to their intrinsic antioxidant properties along with boosting Nrf2, HSP 60, HSP 70 and HSP 90 gene expression levels and as such may be useful potential tools in combating DEHP-induced testicular dysfunction.

Keywords: DEHP; Nrf2; HO-1; HSPs; Resveratrol; Curcumin; Testes; Rat
6- أهم النتائج التطبيقية التي تم التوصل إليها: (لا تزيد عن سطرين لكل منها)

6-1 أعطاء الريسفيراتول أو الكركمين قد أدى إلى الحد من إصابة الخصية الناتج عن مادة ثانوي (إيثيل هكسيل) الفثات.

6-2 تراجع التأثيرات الواقية للريسفيراتول و الكركمين على التأثير المضاد للأكسدة لكل منهما إلى جانب تعزيز التعبير الجيني للـ Nrf2 وكذلك بروتينات الصلادة الحرارية 60 و70 و90 وكذلك السي. كيت بروتين.

7- ما هي الجهات التي يمكن أن تستفيد من هذا البحث:

(تذكر هذه الجهات مع شرح أهمية البحث لهذه الجهه بما لا يزيد عن أربعة سطور لكل جهة)

1- شركات تصنيع الأدوية.

7- استخدام الريسفيراتول و الكركمين في المستحضرات الدوائية المناسبة وذلك بعد استيفائها كافة أنواع الدراسات العملية والإكلينيكية والموافقة على طرحهما كعلاج مساعد للحد من من إصابة الخصية الناتج عن مادة ثانوي (إيثيل هكسيل) الفثات.

8- هل توجد علاقة قائمة بإحدى هذه الجهات:

نعم لا

في حالة نعم ذكر هذه الجهات :

1-8

2-8

3-8

ما هي طبيعة العلاقة :

مشروع بحثي

تعاون أكاديمي

مشروع ممول من جهة ثالثة

(ذكر ما هي :

(ذكر):

9- هل توافق على التعاون مع جهات مستفيدة من خلال الجامعة:

(لماذا )

نعم

(أ) تطبيق البحث:

لا

(ب) لا استكمال البحث:

لا

(ج) أخرى:

(ذكر)
1- Resveratrol and curcumin ameliorate di-(2-ethylhexyl) phthalate induced testicular injury in rats.
Abd El-Fattah AA, Fahim AT, Sadik NA, Ali BM.

2- Poster presentation entitled "Effect of some antioxidants on experimental testicular injury induced in rats" at 5th international scientific conference of faculty of pharmacy Cairo university entitled "pharmacy at the Cutting Edge: Facing Future Challenges" at April 23rd, 24th, 2014

11- هل تم النشر بحوث مستقلة من الرسالة في مجلات أو مؤتمرات علمية? نعم
(تذكر مع جهة النشر و المكان و التاريخ)

لا

12- هل توافق على إعطاء البيانات المذكورة في هذه الاستمارة لجهات أخرى?

نعم

لا

توقيع المشرفين:

توقيع الطالب:

وكل الكلية (المعهد) للدراسات العليا والبحوث:

التاريخ: