Cairo University
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Biochemical effect of some chemical preservatives on reproductive effeciency in male albino rats

A thesis submitted by

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For the degree of the
Ph.D in Veterinary Medicine Science
(Biochemistry and chemistry of nutrition)

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Supervision sheet

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Abstract

Unbalanced exposure to environmental endocrine disruptor compounds during fetal or neonatal life evoked disruption in endocrine function during development. Triclosan and butylparaben are widely used as preservatives and it was reported to have estrogenic activity as EDC. The aim of current study is to address the potential impacts of individual or combined administration of triclosan , butyl paraben on the gonadal hormone and potential combined toxicity representing oxidative stress induction in testicular tissue in weanling male rats. Weanling male rats were orally administered individual or combined butylparaben, triclosan (50 mg $kg^{-1}day^{-1}$) for 4weeks and 8 weeks, followed by determining the serum gonadal hormones, oxidative stress parameters and DNA damage using comet assay in testicular tissues. Results revealed that treated groups compared to control groups caused significant depletion in count and motility of sperm, the relative weight of (SATs) Sex accessories tissue, and levels of (T), (LH), (FSH), T/E_2 and T/LH. Alongside, individual treatment significantly increases (E_2) levels, whereas combined treatment non-significantly decreased its level. All treated groups did not alter the mean differences of both right and left testis relative weights. A significant elevation in oxidative parameters and an obvious testicular DNA damage was recorded. Moreover, a disturbance of antioxidant parameters in all treated groups was observed. Our results revealed that combined treatment aroused an endocrine disturbance activity with a concomitant induction of testicular oxidative stress status which may represent a common mechanism in an endocrine disruptor-mediated dysfunction.

Keywords: Triclosan- Butylparaben- combined toxicity-reproductive toxicity.

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CONTENTS

List of Figures

List of abbreviations

ADP	Adenosine diphosphate
AhR	aryl hydrocarbon
AMA	American Medical Association
APHA	American Public Health Association
ATP	Adenosine triphosphate
BP	Butyl paraben
BPA	Bisphenol A
cAMP	cyclic adenosine monophosphate
CAT	Catalase
CoQ	Coenzyme Q
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid.
DSP	daily sper production
DTNB	5, 5 dithiobis (2-nitrobenzoic acid)
E_2	Estradiol
EDs	Endocrine disruptors
EDCs	Endocrine disruptor chemicals
EDTA	Ethylene diamine tetracetic acid
ER	Estrogen receptor
EROD	ethoxyresorufin Odeethylase

EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin releasing hormone
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	glutathione
GST	Glutathione S-transferase
H&E	Haematocylcine and Eosin stain
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical
hPXR	human pregnane X receptor
IBA	isobutyl paraben
LCs	Leydig cells
LH	Luteinizing hormone
LPO	Lipid peroxidation
MCF-7	Michigan Cancer Foundation-7
MDA	Malondialdehyde
MPT	Mitochondrial Permeability Transition
NADH	Nicotinamide adenine dinucleotide (reduced)
NADP	Nicotinamide adenine dinucleotide phosphate (

	oxidized)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
NaN_3	Sodium azide
NaOH	Sodium hydroxide
NED	N-(1- Naphthyl) ethylenediamine
NO	Nitric oxide
NODCAR	National Organization of Drug Control And Research
NOECs	No observed effect concentrations
NOEL	no observed effect level
NOS	Nitric oxide synthase
$O_2^{\bullet-}$	Superoxide anion
ONOOH	Peroxonitrous acid
$OONO^-$	peroxynitrite
OP	Octyl phenol
PCB	polychlorinated biphenyl
PHBA	p-hydroxybenzoic acid
PROD	pentoxyresorufin O-depentylase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Revolution per minute

SATS	Sex accessories
SC	Sertoli cells
S.D.	Standard deviation
SDH	Succinate dehydrogenase
SHBG	sex/steroid hormone-binding globulin
SOD	Superoxide dismutase
SPSS	Statistical Package for Social Science
STAR	steroidogenic acute protein
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive subestance
TBG	Thyroglobulin
TCDD	2, 3, and 7, 8- tetrachlorodibenzo-p-dioxin
TEBG	testosterone-estrogen-binding globulin
TSC	Triclosan
UGT	UDP- glucuronosyl-transferases
US	United States
USFDA	US Food and Drug Agency
UV	Ultraviolet.
VCl_3	Vanadium chloride
Vtg	vitellogenin

Chapter (1)

Introduction

INTRODUCTION

Based on accumulating evidence, exposure to various endocrine disruptor compounds (EDCs) may disrupt the normal androgen and estrogen balance in animals and humans, potentially leading to sex hormone-sensitive diseases/disorders **(Diamanti-Kandarakis *et al.*,2009; Habauzit *et al.*, 2011).**These phenomena have prompted researchers to address the risks of mixtures of EDCs, where the adverse effects of EDCs may be derived from mixtures of compounds and not from exposure to a single compound. Several in vitro studies discussed the effect of combined mixture of EDCs using different in vitro assay models. The toxic effect of these combinations fluctuated and were synergistic, antagonistic even additive, based on the nature and concentration of EDCs to enhance toxicity **(Payne *et al.*,2001 ;Gray *et al* 2001 ; Charles *et al* 2002; Crofton *et al.*,2005; Kortenkamp.,2007).**

Butyl paraben (BP) is considered a marked preservative in many personal care and pharmaceutical products **(Zhang *et al.*, 2016)** In vivo studies have revealed the controversial estrogenic activity of BP in an immature rodent model. According to research of **Oishi, (2001, 2002[b])** BP is toxic to the reproductive system of immature male mice and rats at doses more than 100 mg/kg bwt. A similar experimental design was conducted by Hoberman and colleagues **(Hoberman *et al.*,2008)** for 3 months using more animals in each group, and the data revealed that BP did not alter reproductive organs. Exposure pregnant Wistar female rats to varying doses of BP indicated that BP act as an estrogenic agent

at a dose of 200 mg/kg bwt, by enhancing aromatase activity to increase synthesis of estrogen hormone, which lead to depletion in testosterone levels in offspring male rats **(Zhang *et al*.,2014)** Paradoxically, **Kang *et al*.(2002)** revealed that BP at doses of 100 or 200 mg/kg bwt did not change the reproductive organs in offspring female rat. Triclosan is a potent antimicrobial agent that is widely used as preservative in personal care products, plastics, and fabrics **(Black and Howes ,1975 and Zorrilla *et al*.,2009).**

In vivo study for estrogenic activity of triclosan showed that it potentially induced vitellogenin expression in male medaka while decreasing the hatchability and delaying the hatching in females **(Ishibashi *et al*.,2004).** Moreover, exposure of japanese rice fish (Oryzias latipes) also known as the medaka, to different doses of triclosan or 17βestradiol did not support the hypothesis that triclosan has estrogenic activity, and thus triclosan is believed to be a potential weak androgenic agent**.(Foran *et al*.,2000)**

Zorrilla *et al.*, (2009) revealed that oral administration of triclosan with different doses did not affect the onset of preputial separation (PPS), where testosterone levels were significantly decreased only at a dose of 200 mg/kg. Conversely, **Feng *et al.*,(2009)** revealed that administration of triclosan, with different doses to pregnant rats, from gestation day 6 to gestation day 20, decreased offspring uterine weight. Furthermore, it induced a depletion in human chorionic gonadotropin hormone, E2, Testosterone hormone levels as well as prolactin, while it did not alter both follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels only at 600 mg/kg