BIOCHEMICAL STUDIES ON NANOPARTICLES AS ANTIOXIDANT AGENTS

By

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B.Sc. Agric. Sci. (Agricultural Biochemistry), Fac. Agric., Cairo Univ., 2007
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APPROVAL SHEET

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ABSTRACT

The present study was undertaken to evaluate the effects of chitosan (CS) and chitosan nanoparticles (CSN) on rats suffering from dimethoate (DM) and evaluate the effects of CS and CSN on human cancerous cell lines (MCF7, HCT116, A549 and PC3).

CSN were prepared based on the ionic gelation of chitosan with tripolyphosphate anions. The physicochemical properties of the nanoparticles were determined by size and zeta potential analysis, Transmission Electron Microscopy (TEM) and Fourier Transform Infrared analysis (FTIR). The average diameter and zeta potential of CSN were 116.5 nm and 6.43 mV, respectively. Subsequently, the cytotoxicity of CS and CSN were examined by the methyl tetrazolium (MTT) assay. CSN showed the anticancer activity with the LC_{50} values of 101.28 µg/mL in MCF7, 367.65 µg/mL in PC3, 666.67 µg/mL in HCT116 and 681.82 µg/mL in A549, whereas CS gave the LC_{50} values of 102.67 µg/mL in MCF7, 694.44 µg/mL in PC3, 1470.59 µg/mL in HCT116 and 769.23 µg/mL in A549.

The male albino rats treated with CS (500 mg/kg bw), CSN (250 mg/kg bw) and combination between CS (250 mg/kg bw) and CSN (125 mg/kg bw) along with administration of DM (1% DM/corn oil; 20 mg/kg bw) for 3 weeks. The obtained results showed that the treatments generally lowered the DM-induced activities of hepatic enzyme markers (AST, ALT, ALP and LDH), kidneys function parameters levels (urea, uric acid and creatinine) and lipid profile levels (triglycerides, cholesterol, LDL-C and VLDL-C) but increased protein profile (protein, albumin and globulin) and HDL-C levels. Also, the histopathological examination showed marked improvements in histological structure of the liver.

Therefore, the results of this study suggest that CS and CSN may be potentially used as anticancer against MCF7, HCT116, A549 and PC3 cell lines. In addition to the protective effect of CS and CSN adult rats against the DM-induced damage in rats. Also, chitosan did not show any toxicity on normal rats.

Key words: Cancer cell lines, chitosan, chitosan nanoparticles, dimethoate, kidneys function, liver function.

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LIST OF ABBREVIATIONS

No.	Abbreviation	
1.	4-AAP	4-aminoantipyrine
2.	AChE	Acetylcholinesterase
3.	ADP	Adenosine diphosphate
4.	ALP	Alkaline phosphatase
5.	ALT	Alanine aminotransaferase
6.	AST	Aspartate aminotransaferase
7.	ATP	Adenosine triphosphate
8.	BNPP	bis(4-nitriphenyl)phosphate
9.	CAT	Catalase
10.	CS	Chitosan
11.	CSN	Chitosan nanoparticles
12.	DD	Degree of deacetylation
13.	DLS	Dynamic Light Scattering
14.	DM	Dimethoate
15.	DMSO	dimethylsulfoxide
16.	DPPH	2, 2̀- diphenyl – 1- picrylhydrazyl
17.	E	Eosin
18.	EDTA	Ethylenediaminetetraacetic acid
19.	FAO	Food and Agriculture Organization
20.	FTIR	Fourier Transform Infrared
21.	GD	Gestation days
22.	GK	glycerol kinase
23.	GOD	Glucose oxidase
24.	GPO	glycerol phosphate oxidase
25.	GPx	Glutathion peroxidase
26.	H	Heamtoxylin
27.	HDL-C	High density lipoprotein-cholesterol
28.	ICR	Imprinting Control Region
29.	LC	Lethal concentration
30.	LDL-C	Low density lipoprotein-cholesterol
31.	LPL	lipoprotein lipase
32.	LPO	Lipid peroxidation
33.	LSD	Least significant difference
34.	MFO	Mixed function oxidase
35.	MMP	Mitochondrial membrane potential

36.	MPS	Mononuclear phagocyte system
37.	MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
38.	NPs	Nanoparticles
39.	NRC	National Research Center
40.	OPI	Organophosphate pesticides
41.	PEG	Polyethylene glycol
42.	POD	peroxidase
43.	ROS	Reactive oxygen species
44.	SDS	Sodium dodecyl sulphate
45.	SEM	scanning electron microscope
46.	SNPs	Solid nanoparticles
47.	SOD	Soperoxide dismutase
48.	STAR	Steroidogenic acute regulatory
49.	TBARS	Thiobarbituric acid – reactive substances
50.	TEM	Transmission electron microscopy
51.	TPP	Tripolyphosphate
52.	VLDL-C	Very low density lipoprotein-cholesterol
53.	WHO	World Health Organization

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INTRODUCTION

Nanotechnology, an interdisciplinary research field involving chemistry, engineering, biology, and medicine, has great potential for early detection, accurate diagnosis, and personalized treatment of cancer (Cai and Chen, 2007). Nanoparticles are typically smaller than several hundred nanometers in size, comparable to large biological molecules such as enzymes, receptors, and antibodies. With the size of about one hundred to ten thousand times smaller than human cells, these nanoparticles can offer unprecedented interactions with biomolecules on both the surface of and inside the cells, which may revolutionize cancer diagnosis and treatment. The most well-studied nanoparticles include quantum dots (Cai *et al.,* 2006 and 2007), carbon nanotubes (Liu *et al.,* 2007), paramagnetic nanoparticles (Thorek *et al.,* 2006), liposomes (Park *et al.,* 2004b), gold nanoparticles (Huang *et al.,* 2007), and many others (Ferrari, 2005; Grodzinski *et al.,* 2006).

Chitosan (CS) is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. It is one of the major cationic polymers and the second most abundant polysaccharides in nature (Choi *et al.,* 2016). The $–NH_2$ and –OH groups endow CS with many special properties, making it applicable in many areas and easily available for chemical reactions. CS is safe, non-toxic and can interact with polyanions to form complexes and gels (Sunil *et al.,* 2004; Se and Niranjan, 2005). The use of natural compounds with chemopreventive properties has attracted much interest in chemotherapy and treatment of cancers. A good example is

the use of CS, a naturally occurring polymer that has been often tested in pharmaceutical and biomedical applications because of its promising properties such as biocompatibility, biodegradability and lower toxicity towards mammalian cells (Rinaudo, 2006). CS was found to have various biological activities including antimicrobial activity (Younes *et al.*, 2012), antioxidant activity (Younes *et al.*, 2014), CS is also reported to have anti-tumor effects by inhibiting tumor cell proliferation (Maeda and Kimura, 2004), inducing apoptosis (Pae *et al.*, 2001), and enhancing immune functions (Yu *et al.*, 2004).

Chitosan nanoparticles (CSN) may exhibit potential antibacterial activity as their unique character (Qi *et al.*, 2004). The unique character of nanoparticles could make CSN exhibit more superior activities than chitosan. CSN have been reported to have heightened immune-enhancing effect (Wen *et al.*, 2011), anticancer activity (Qi *et al.*, 2007) and antimicrobial activity of chitosan (Qi *et al.*, 2004).

Cancer remains to be one of the leading causes of death worldwide. Over the past several decades, significant advancements have been made in fundamental understanding of cancer biology, which has in turn led to better diagnostic and treatment methods. A major reason for this is our inability to administer therapeutic agents selectively to the targeted sites without adverse effects on healthy tissue. Current therapeutic strategies for most cancers involve a combination of surgical resection, radiation therapy, and chemotherapy (Ruoslahti *et al.*, 2010). Conventional chemotherapy can wreak havoc on healthy tissue, causing painful side effects, and it is not always effective. At the same time, there is the huge risk that the drugs