INVESTIGATION OF SOME BIOLOGICAL ACTIVITIES OF WATER SOLUBLE CHITOSAN

Hnin Wuit Yee[1], Daw Hla Ngwe[2]

Abstract

The main aim of the research is to study the deacetylation of chitin extracted from prawn shell by enzymatic method and to evaluate scientifically on some biological activities. The isolation of chitin deacetylase producing *Bacillus thermoleovorans* from soil and bioconversion of chitin to chitosan and water soluble chitosan (WS-chitosan) was converted from the prepared E-chitosan by oxidative depolymerization method. Some pharmacological activities such as antimicrobial activity, antitumor activity, antioxidant activity, acute toxicity, weight loss activity and antilipidemic activity of water soluble chitosan (WS-chitosan) were currently assayed. The antimicrobial activity of WS-chitosan was more effective on gram positive bacteria than gram negative bacteria. The antitumor activity of WS-chitosan was assayed by potato crown gall (PCG) method. It was found that, the different concentration ranges of chitosan have different inhibited tumor growth manner. In the determination of antioxidant activity of WS-chitosan, the IC_{50} was found to be 4.20 µg/mL. The acute toxicity test of WS-chitosan on albino mice done by the method of the Litchfield and Wilcoxon indicated no toxic effect. According to the study on the weight loss activity of WS-chitosan on albino mice carried out by the method of Han *et al*., 1999, the 600 mg/kg b. wt dose of WS-chitosan is the most eligible dose to reduce the body weight of male and female albino mice. In the investigation of antilipidemic activity of WS-chitosan on albino rats, it was observed the significantly decrease in the "bad" cholesterol: LDL cholesterol and increase in "good"cholesterol : HDL cholesterol. Treatment with WS-chitosan (600mg/kg b.wt) significantly decreased in levels at TC by 59 % , TG by 62 % and LDL by 40 % and 26 % increased in HDL levels at 21 days.

Keywords : water soluble chitosan, antimicrobial, antitumor, antioxidant, weight loss activity, antilipidemic activity

Introduction

Chitosan is a natural linear polysaccharides comprising copolymers of glucosamine and N-acetyl glucosamine and can be obtained by the partial deacetylation of chitin. Nowadays, chitosan is used in versatile applications

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(Choi *et al*., 2004). Chitosan has been widely used in vastly diverse fields ranging from waste management to food processing, medicine and biotechnology. It becomes an interesting material in pharmaceutical applications due to its biodegradability and biocompatibility, and low toxicity.

Water soluble chitosan (WS-chitosan) was prepared from enzymatically prepared chitosan (E-chitosan) that was produced by using chitin deacetylase enzyme (CDA) extracted from *B. thermoleovorans* that was isolated from soil sample (Hnin Wuit Yee *et al.,* 2016). In the present study, some biological activities such as antimicrobial, antioxidant, antitumor, weight loss and antilipidemic activities of the water soluble chitosan were investigated.

Antimicrobial activity: Chitosan is effective in inhibiting growth of bacteria but lower toxicity towards mammalian cells (Takemone *et al*., 1989). The antimicrobial properties of chitosan depend on its molecular weight and the type of bacterium, chitosan generally showed stronger bacterial effects for gram-positive bacteria, (*Listeria monocytogenes, Lactobacillus plantarum, L.brevis, Bacillus cereus, Staphylococcus aureus, Bacillus subtilis and Bacillus pumilus*) than for gram-negative bacteria (*Pseudomonas aeruginosa, Pseudomonas fluorescens, E. coli, Salmonella typhymurium and Vibrio parahaemolyticus*) in the presence of 0.1 % chitosan (No *et al*., 2002).

Antitumor activity: The use of highly specific, quantitative bioassays which require only a short period of time to obtain results is available for studying crown gall tumor formation (Anand and Heberlein, 1977). The crown gall tumor assay (CGTA) is one of several bench top bioassays recommended for the rapid screening of plants with anti-cancer activity (Galsky *et al*., 1980 and Srirama *et al*., 2008).

Antioxidant activity: Antioxidant activity is one of the well-known functions of chitosan. Many studies have shown that chitosan inhibit the reactive oxygen species (ROS) and prevent the lipid oxidation in food and biological systems (Kim and Thomas, 2007). Chitosan can scavenge free radicals or chelate metal ions from the donation of a hydrogen or the lone pairs of electrons (Xie *et al*., 2001). The interaction of chitosan with metal ions could involve several complex actions including adsorption, ion-exchange and chelation (Onsosyen and Skaugrud, 1990). The hydroxyl group (OH) and amino groups (NH_2) in

chitosan are the key functional groups for its antioxidant activity but can be difficult to be dissociated due to the semi-crystalline structure of chitosan with strong hydrogen bonds (Xie *et al*., 2001). There has been increasing interest in finding natural antioxidants, since they can protect the human body from free radicals and retard the progress of many chronic diseases.

Weight loss activity: Chitosan is viewed as potentially useful for increase fecal fat excretion. This effect has been demonstrated in animal studies. Several studies have examined whether chitosan consumption will reduce adiposity in animal models, as might be expected, based on the increase in fecal fat. None the less, in human studies, the chitosan with low molecular weight can accelerate the weight loss when subjects are on a weight reduction diet, but that when food intake is not restricted, no weight loss should be expected.

Antilipidemic activity: Chitosan is believed to affect cholesterol levels and weight because it has positively charged amino groups at the same pH as the gastrointestinal tract. These amino groups are believed to bind to negatively charged molecules, such as lipids and bile, preventing their absorption and storage by the body. The action of chitosan in cholesterol management may be explained by the theory that ingested chitosan salts react with fatty acids and bind lipids because of hydrophobic interactions; these bound lipids are extracted rather than absorbed (Gallaher *et al*., 2000).

Materials and Methods

Screening of Pharmacological Activities of Prepared Water Soluble Chitosan

The isolation of chitin deacetylase producing *Bacillus thermoleovorans* from soil and bioconversion of chitin and chitosan and water soluble chitosan (WS-chitosan) was converted form the enzymatically prepared chitosan (E-chitosan) by oxidative depolymerization method were previously described (Hnin Wuit Yee *et al.,* 2016). Some pharmacological activities of water soluble chitosan chitosan such as antimicrobial activity, antitumor activity, antioxidant activity, acute toxicity, weight loss activity and antilipidemic activity were presently studied.

Screening of antimicrobial activity by agar well diffusion method

Antimicrobial activity of various concentration of prepared water soluble chitosan (WS-chitosan) was studied by agar well diffusion method at Pharmaceutical Research Department, Insein, Yangon Region. The agar well plate diffusion method was used to test the antibacterial action of the extracts on 24 h broth culture of the organisms used. The prepared water soluble chitosan (WS-chitosan) in various concentrations such as 1.0 mg, 2.0 mg and 3.0 mg were dissolved in 1mL of sterile distilled water. 1 mL each of the bacterial suspension of 24 h of nutrient agar was streaked evenly onto the surface of trypticase soy agar plates with sterile cotton swab. Immediately after hardening the agar, the wells were made with a 10 mm sterile cork borer from each seeded agar. After removing the agar, the wells were filled with the WS-chitosan to be tested. The plates were incubated at 37 °C for 18 - 24 h. The diameters of the inhibition zone were measured and recorded in mm.

Screening of antitumor activity by potato crown gall test

Isolated *Agrobacterium tumefacien* has been maintained as solid slants under refrigeration. For inoculation into the potato discs, 48 h broth cultures containing 5×10^7-5×10^9 cell/mL were used. Fresh, disease free potatoes were purchased from a local market. Tubers of moderate size were surface sterilized by immersion in 0.1 % sodium hypochlorite for 20 min. Ends were removed and the potatoes were soaked an additional 10 minutes. A core of the tissue was extracted from each end and discarded. The remainder of the cylinder was cut into 0.5 cm thick discs with a surface sterilized scalpel. The discs were then transferred to agar plates (1.5 g of agar dissolved in 100 mL sterile distilled water (DW), autoclaved for 20 min at 121°C, 20 mL poured into each petri-dish). Each plate contained four potato discs and 4 plates were used for each sample dilution.

Each sample of 2.50, 1.25 and 0.625 mg was separately dissolved in 2 mL of Hexodeutrodimethyl sulphoxide (DMSO) and filtered through millipore filters (0.22 μm) into sterile tube. This solution (0.5 mL) was added to sterile DW (1.5 mL), and broth culture of *A. tumefaciens* in 2 mL of phosphate buffer saline (PBS) was added. Control was made in this way; DMSO (0.5 mL) and sterile DW (1.5 mL) were added to the tube containing 2 mL of broth culture

of *A. tumefaciens*. Using a sterile disposable pipette, 1 drop (0.05 mL) from these tube was used to inoculate each potato disc, spreading it over the disc surface. After inoculation, Petri dishes were sealed by para film and incubated at 27 - 30 °C for 3 weeks. Tumors were observed on potato discs after 21 days under stereo-microscope followed by staining with Lugol's iodine (10 % KI and 5 % I_2) after 30 minutes and compared with control. The antitumor activity was examined by observation of tumor produced or not. The steps involved in the preparation of potato discs for PCG test were shown in Figure 1.

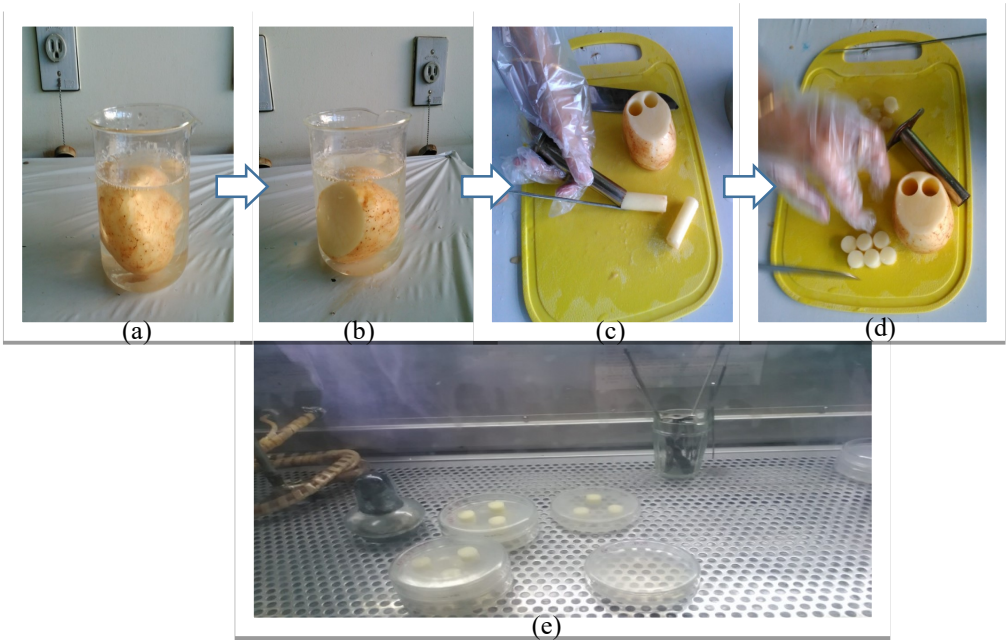


Figure 1. Photographs showing the steps involved in the preparation of potato discs for PCG test

(a) Sterilized potato tuber surface by immersion in 0.1 % sodium hypochlorite solution
(b) Removed both ends of potato tuber surface by sterilized cutter and soaked in another 0.1 % sodium hypochlorite solution
(c) Extracted the core of potato with sterilized cork borer
(d) Cut the core of potato into 0.5 cum thick discs with a sterilized scalpel
(e) Transferred the potato discs to petridish containing agar medium

Determination of antioxidant activity by using DPPH assay

DPPH radical scavenging activity was determined by UV visible spectrophotometric method. Control solution was prepared by mixing 60 μM DPPH solution (1.5 mL) and 70 % ethanol (1.5 mL). The sample solution was prepared by thoroughly mixing 60 μM DPPH solution (1.5 mL) and test sample solutions (1.5 mL). The solution was then allowed to stand at room temperature for 30 minutes. Absorbance of these solutions was measured at 517 nm by using spectrophotometer and % inhibition was calculated by using the following equation. The 50 % inhibition concentration (IC_{50}) value for each test sample was determined by linear regressive excel programme from a plot of % inhibition values versus the concentration of each test sample.

$$\% \text{ Inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Where, $A_{Control}$ = absorbance of control solution

A_{Sample} = absorbance of tested sample solution.

Study on acute toxicity of water soluble chitosan

The acute toxicity test on prepared water soluble chitosan was done according to the method of Litchfield and Wilcoxon (1949). Fifty healthy albino mice (ddy strain) of both sexes (30-35) g were used for this study. They were separated into 6 groups, each group containing of 10 mice. On the experiment day, all groups of mice were fasted overnight and only water was taken. Four doses (1 g/kg, 2 g/kg, 3 g/kg, 4 g/kg and 5 g/kg) of prepared water soluble chitosan were administered orally. The mice of control group were given distilled water only. The general signs and symptoms of toxicity were recorded hourly up to 6 hours and daily up to two weeks (Litchfield and Wilcoxon, 1949). The procedure steps of acute toxicity test on albino mice are illustrated in Figure 2.

(a) **(b)**

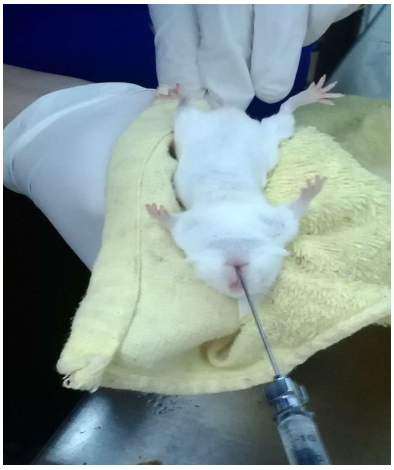
Figure 2. Acute toxicity test on albino mice (DDY strain)

(a) Mice were put in laboratory condition: standardized boxes, natural light and ambient temperature, allowed free access to both water and animal feed

(b) Administration of the prepared WS-chitosan by oral route using intragastric syringe

Weight loss effect of prepared water soluble chitosan (WS-chitosan) in mice

The weight loss activity of WS-chitosan tested on albino mice was carried out according to the method of Han *et al*., (1999). Eighty healthy albino mice (ddy strain) of male and female (32-35 g) were used for this study. They were separated into 4 groups, each group containing of 10 mice. Group I was given distilled water only and served as control group. Groups II, III and IV were orally given with three different doses (400, 600 and 800 mg/kg) of prepared sample respectively and orally administered once a day for 4 weeks as shown in Figure 3.



(a) Administration of the prepared WS-chitosan in different concentrations by oral route using intragastric syringe

(b) Checking the weight of tested mice by using animal balance

Figure 3. Weight loss test on albino mice (DDY strain)

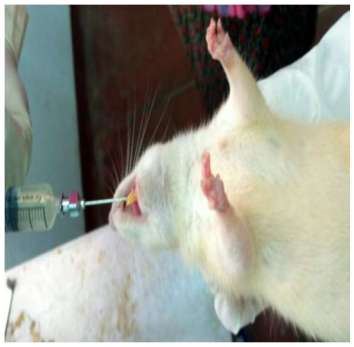
Antilipidemic effect of prepared water soluble chitosan

All rats were weighed before the experiment. The dosages were calculated on the body weight basis for each tested rat. Thirty female albino rats were divided into five groups, each group containing of 6 animals. Hyperlipidemia was induced in rats by single intraperitoneal injection of freshly prepared solution of triton WR-1339 (400 mg/kg) in physiological saline solution after the animals were kept fasting for 24 h (Sikarwar and Patil, 2012).

Each animal was given single dose of triton 400 mg/kg intraperitoneally shown in Figure 4 (a). The Group I was given distilled water only and served as control group. After 48 h of triton injection, Group II was orally treated with atorvastatin 10 mg/kg and served as a standard drug treated group. Groups III, IV and V were orally given with three different doses (400, 500, 600 mg/kg) of prepared WS-chitosan respectively after 48 hours of triton

injection. Atorvastatin and three different doses of WS-chitosan were orally administered once a day for 4 weeks (Figure 4). The plasma lipid levels (TC, TG, HDL, LDL) of each rat were measured at the start of the study i.e., before triton injection (For base line plasma lipid level) 48 hours after triton injection (to confirm triton induced hyperlipidemia), 2 days, 7 days, 14 days and 21 days after orally administered by samples and standard drug for all respective groups. The photographs showing the measurement steps of plasma lipid level are shown in Figure 5.

(a)



(b)

(c)

Figure 4. Steps involved in the antilipidemic test on albino rats (Wistar strain)

(a) Intraperitoneal injection of triton (400mg/kg) to albino rats

(b) Administered the Atorvastatin (10mg/kg) to albino rats by oral route using intragastric syringe

(c) Administered the prepared WS-chitosan to albino rats by oral route using intragastric syringe

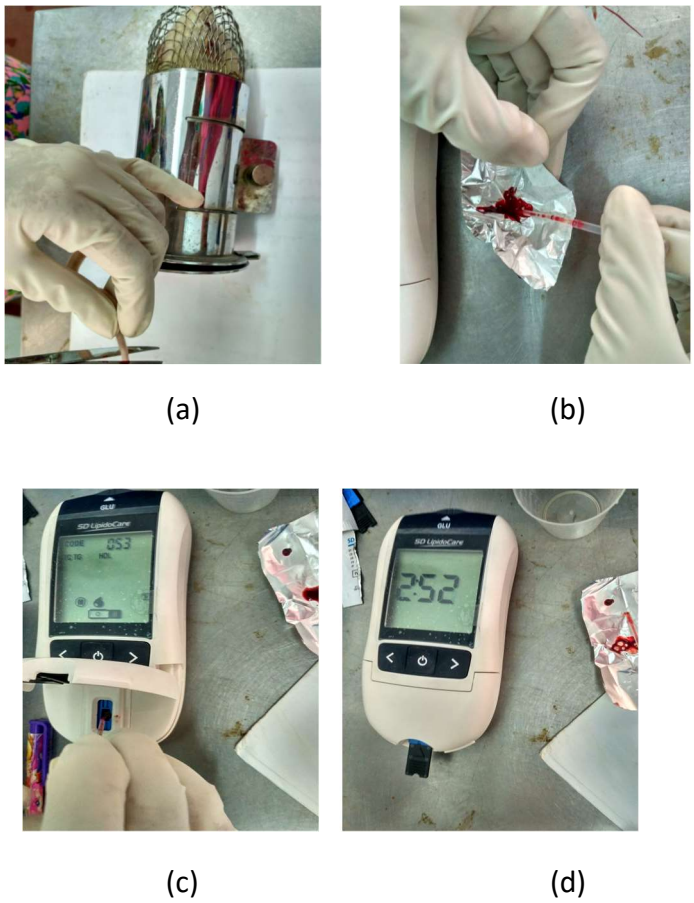


Figure 5. Photographs showing the steps involved in the measurement of plasma lipid levels (TC, TG, HDL, LDL) of each rat by using "SD Lipidocare" device

(a) Cut the rat tail to collect the blood by sterilized scissors

(b) Take the rat blood about 30 µL with capillary tube

(c) Plugged the test strip in the SD Lipidocare device and put the rat blood sufficiently on the sensor of this device

(d) Read the data of plasma lipid levels (TC, TG, HDL, LDL) by choosing the mode

RESULTS AND DISCUSSION

Pharmacological Activity of Prepared Water Soluble Chitosan

In vitro screening of antimicrobial activity of prepared WS-chitosan by agar well diffusion method

From the results shown in Figure 6, it was observed that 2.0 mg concentration showed slightly higher activity than other (1 mg and 3.0 mg) in 1 mL of sterile distilled water. According to the investigation of antimicrobial activities on tested microorganisms, the prepared water soluble chitosan showed stronger antimicrobial effects for gram positive bacteria such as *B.subtilis, Staphylococcus aureus* and *B.pumilus* than others. Diameters of inhibition zone (mm) for tested samples are summarized in Table 1. The inhibition zone diameters of tested samples against seven microorganisms were found in the range of 15-18 mm.

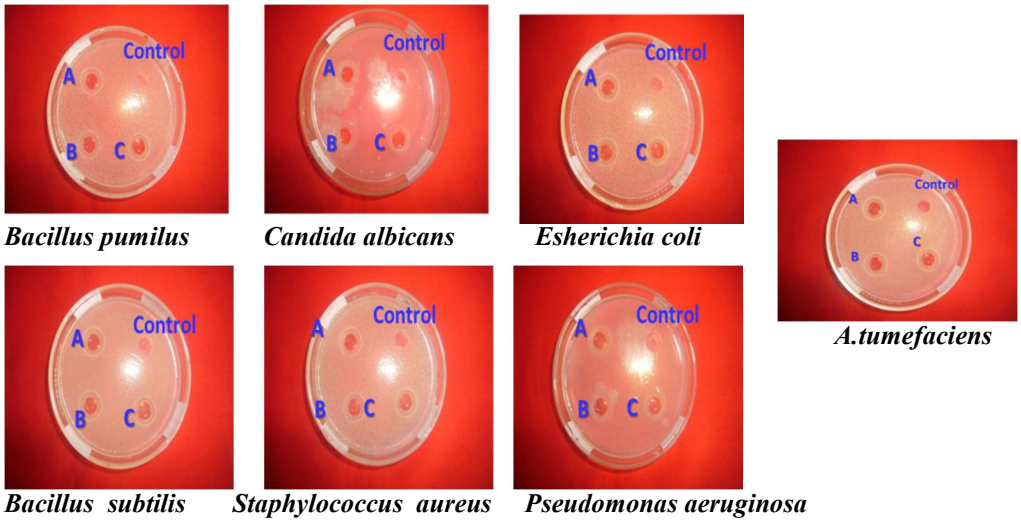


Figure 6. Screening of antimicrobial activity of prepared WS-chitosan against six microorganisms and *A. tumefaciens*

Table 1. Inhibition Zone Diameters of Prepared WS-chitosan in Different Concentrations

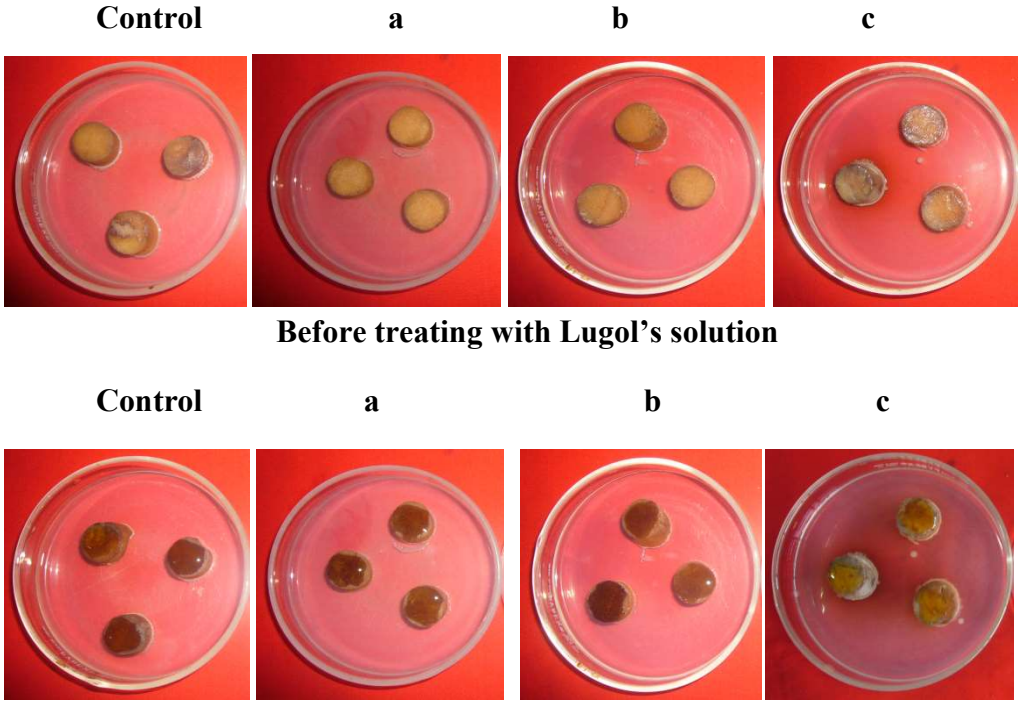
Samples	Concentration (mg/mL)	Diameters of inhibition zone (mm) against different microorganisms						
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**
WS – chitosan in distilled water	1.0	17 (++)	16 (++)	15 (++)	16 (++)	16 (++)	16 (++)	16 (++)
	2.0	18 (++)	18 (++)	15 (++)	18 (++)	15 (++)	16 (++)	17 (++)
	3.0	17 (++)	17 (++)	15 (++)	16 (++)	15 (++)	15 (++)	17 (++)
Control (D/W)	-	-	-	-	-	-	-	-

I	*Bacillus subtilis*	Agar Well – 10 mm
II	*Staphylococcus aureus*	10 mm ~ 14 mm (+)- lower activity
III	*Pseudomonas aeruginosa*	15 mm ~ 19 mm (++) - higher activity
IV	*Bacillus pumilus*	20 mm ~ above (+++) - highest activity
V	*Candida albicans*	
VI	*Escherichia coli*	
VII	*A. tumefaciens*	

Antitumor activity of prepared water soluble chitosan (WS-chitosan)

It could be seen from the Figure 7 that the low molecular weight water soluble chitosan (WS-chitosan) inhibited tumor growth in concentration dependent manner. Significant tumor inhibition was observed at 7.81 µg/disc and slightly inhibited on tumor growth at 3.90 µg/disc, but not at 1.95 µg/disc. Hussian *et al* (2007) have also shown that inhibition rate on potato discs are dependent on concentration of chitosan and also tumor producing *A. tumefaciens* strains. It was noticed that tumor formation was observed when *Agrobacterium* strains alive on potato disc. The potato discs were often

damaged due to the contamination and other physiological factors when there was no tumor formation. Thus successful attachment of *Agrobacterium* on living potato disc is needed for antitumor test. The results of antitumor activity are presented in Table 2.



a = 7.81 µg/disc **b** = 3.90 µg/disc **c** = 1.95 µg/disc

Figure 7. Screening of tumor inhibition of WS-chitosan on potato discs in different concentrations

Table 2. Tumor Inhibition by the WS- chitosan through *A. tumefaciens* Infection using Potato Disc Bioassay

Test Sample	**Concentration (µg/disc)**	**Tumor**
WS-Chitosan	7.81	-
	3.90	-
	1.95	+
Control (D/W)	-	+

(+) tumor appeared (-) no tumor appeared

Antioxidant activity of low molecular weight water soluble chitosan (WS-chitosan)

DPPH (2, 2-diphenyl -1, picryl hydrazyl) method is the most widely reported method for screening of antioxidant activity on many organic drugs. This method is based on the reduction of color of free radical DPPH in ethanol solution by different concentrations of the samples. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}). In this study, ten different concentrations (0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 µg/mL) of prepared WS-chitosan were used. Determination of absorbance was carried out at wavelengths 517 nm using UV visible spectrophotometer. Each experiment was done triplicate. The lower the IC_{50} showed the higher the free radical scavenging activity. The IC_{50} values was found to be 4.20 µg/mL for water soluble chitosan which was lower than standard ascorbic acid (IC_{50} =1.43 µg/mL) in antioxidant activity presented in Figure 8.

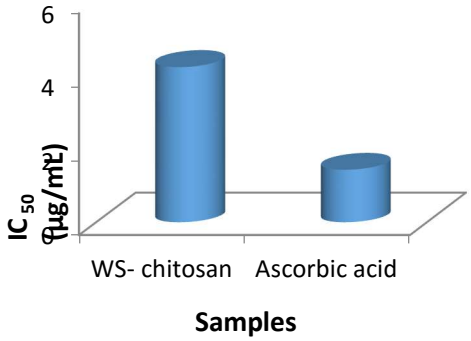


Figure 8. A bar graph representing IC_{50} values of water soluble chitosan and standard ascorbic acid

Acute toxicity study of water soluble chitosan (WS-chitosan)

In this research work, the acute toxicity of water soluble chitosan (WS-chitosan) was determined by Litchfield and Wilcoxon method (1949). Since the route of administration selected should be intended route for administration of tested drugs to human during therapy, the oral route was chosen and different doses were administered orally in mice. The mice weighing 30-35 g were treated with 1, 2, 3, 4 and 5 g/kg body weight doses of each extract. They

were then kept under observation for two weeks. All of the animals were observed to be remained alive and did not show any visible symptom of toxicity like restlessness, respiratory disorders, convulsions, aggressive activities, coma and death at the dosages tested. According to the results as shown in Table 3, no lethality of the mice was observed up to two weeks, even with the maximum soluble dose of 5 g/kg body weight. From these results, it was found that the prepared WS-Chitosan was free from acute toxic effect under condition.

Table 3. Results of Acute Toxicity Test of Water Soluble Chitosan on Mice after Two Weeks Treatment

No	Groups	Drug Administration	Dosage g/kg (b. wt)	No. of death per tested mice	% of death
1.	Control	Distilled Water	-	0/10	0
2.	I	Water soluble chitosan	1	0/10	0
3.	II	Water soluble chitosan	2	0/10	0
4.	III	Water soluble chitosan	3	0/10	0
5.	IV	Water soluble chitosan	4	0/10	0
6.	V	Water soluble chitosan	5	0/10	0

Note : Each group contains 10 no: of mice

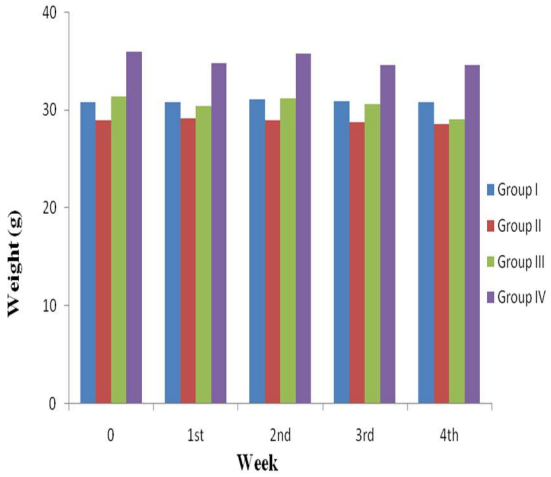
Medium lethal dose LD_{50}> 5 g/kg body weight

Weight loss effect of water soluble chitosan

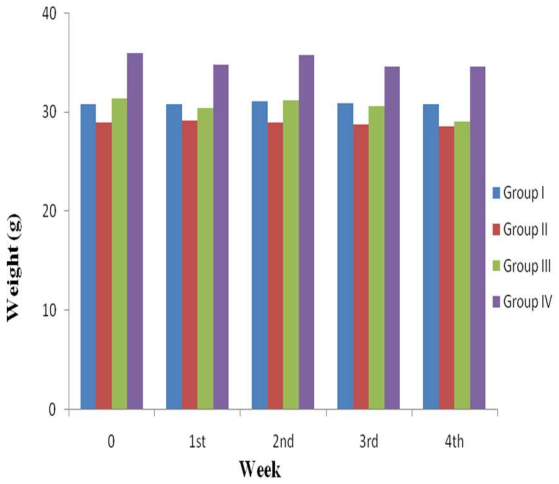
In this research, the weight loss activity of WS-chitosan tested on albino mice by Han *et al.,* 1999. In this test, 80 healthy albino mice (32-40 g b.wt) of male and female were used. They are subdivided into four main groups. Each group contains10 male mice and 10 female mice. The group I was the control group and it was given distilled water only. Groups II, III, IV were test groups treated with three different doses (400 mg/kg, 600 mg/kg and

800 mg/kg) of water soluble chitosan, respectively. They were orally administered once a day for 4 weeks.

In Group II, water soluble chitosan (400 mg/kg body weight) treated group is slightly decreased in third week and in fourth week compared to "0" week. In fourth week, weight loss (29.0 g to 28.6 g) decreased in male albino mice and (29.7 g to 26.0 g) in female albino mice were observed. WS-chitosan possessed more weight loss effect on female albino mice than male albino mice by the above data (Figures 9 (a) and (b)). In the WS-chitosan (600 mg/kg b.wt) treated group III, the weight loss activity was significantly high at fourth week compared to "0" week, 31.4 g reduced to 29.1 g in male albino mice and 31.0 g reduced to 27.3 g in female albino mice. In the water soluble chitosan (WS-chitosan), 800 mg/kg body weight treated Group IV, the weight loss activity showed high at third week compared to "0" week, 36.0 g lowered to 34.6 g in male and 30.8 g lowered to 28.5 g in female albino mice. According to the data, (600 mg/kg body weight) dose of WS-chitosan showed the highest activity than others. Comparison of weight loss activity of different concentrations of WS-chitosan with control group of male and female albino mice is shown in Figure 9.



(a) Male



(b) Female

Group I = Control group treated with D/W only
Group II = The Group treated with 400 mg/kg body weight dose of WS-chitosan
Group III = The Group treated with 600 mg/kg body weight dose of WS-chitosan
Group IV = The Group treated with 800 mg/kg body weight dose of WS-chitosan

Figure 9. Comparison of weight loss activity treated with three different doses of WS-chitosan and control group of (a) male and (b) female albino mice in each week

Study on antilipidemic activity of prepared water soluble chitosan

In hyperlipidemic activity, 30 female albino rats in the weight range of 250-300 grams were used in which 18 rats treated with prepared water soluble chitosan. Thirty albino rats were divided into five groups, each group

containing of 6 animals. Each animal was given a single dose of a triton at a dose of 400 mg/kg b.wt intraperitoneally. The Group I animals were injected triton only and served as control group. After 48 h of triton injection, Group II was orally treated with standard drug (Atorvastain) 10 mg/kg b.wt daily and served orally doses of (400, 500, 600 mg/kg b. wt) of the prepared water soluble chitosan powders, respectively, orally after 48 h of triton injection in Groups III, IV, V. The plasma lipid levels (TC, TG, HDL and LDL ratio) of each rat were measured by SD "Lipidocare" lipid profile at the start of the study i.e., before triton injection (for base line plasma lipid level), 2 days, 7 days, 14 days and 21 days after triton injection (to confirm induced hyperlipidemia) for all groups.

The mean serum levels of base line lipid profiles at "0" hour

The mean serum levels of total cholesterol at 0 h in Groups I, II, III, IV and V were 121.1 ± 27.99 mg/dL, 124.1 ± 28.11 mg/dL, 123.7 ± 30.94 mg/dL, 115.0 ± 17.10 mg/dL and 107.0 ± 31.11 mg/dL respectively. The mean serum levels of triglycerides at 0 h in Groups I, II, III, IV and V were 81.3 ± 11.31, 104.8 ± 21.87, 114.0 ± 11.32, 94.0 ± 21.11 and 75.4 ± 31.50 mg/dL, respectively. The mean serum levels of HDL at 0 hour in Groups I, II, III, IV and V were 57.8 ± 11.20, 70.8 ± 17.10, 75.8 ± 20.81, 77.0 ± 15.99 and 63.5 ± 15.28 mg/dL, respectively. The mean serum levels of LDL at 0 hour in Groups I, II, III, IV and V were 46.6 ± 12.27, 33.0 ± 11.27, 25.5 ± 15.81, 19.3 ± 11.10 and 27.7 ± 6.11 mg/dL, respectively. The resultant lipid profile status of all group of rats at 0 hour of observation were not significantly different ($P< 0.05$). Therefore, the baseline values were same and comparable.

Effect of triton on lipid profiles of triton induced albino rats

The lipid profile observed at 0 h before administration of triton (400 mg/kg b.wt) in Group I were found to be TC: 121.12 ± 27.9 mg/dL, TG: 81.3 ± 11.31 mg/dL, HDL: 57.8 ± 11.2 mg/dL and LDL: 46.6 ± 12.2 mg/dL. The lipid profiles found at 2 days after administration with triton (400 mg/kg b.wt) dose in Group I were increased in TC: 223%, TG: 390 %, LDL: 207 % and HDL: 7 %. The lipid profiles found at 7 days after administration with triton (400 mg/kg b.wt) dose in Group I were increased in TC: 214 %, TG: 377 %, LDL: 199 % and HDL: 3 %. The lipid profiles found at 14 days after administration

with triton (400 mg/kg b.wt) dose in Group I were increased in TC: 134 %, TG: 312 %, LDL: 178 % and HDL: 3 %. The lipid profiles found at 21 days after administration with triton (400 mg/kg b.wt) dose in Group I were increased in TC: 89 %, TG: 303 %, LDL: 163 % and HDL: 2 %. The mean serum levels of TC, TG and LDL observed at 7 days were significantly increased from those observed at 0 hour in the same group ($p<0.05$). Therefore, triton 400 mg/kg can be successfully induced hyperlipidemia in albino rats. These results are presented in Table 4.

Table 4. Mean Plasma Lipid Levels of Group I Receiving Triton 400 mg/kg b.wt dose as Control Group after 2 days, 7 days, 14 days and 21 days

Mean Plasma Lipid Levels (mg/dL)	**Lipid Profile (mean ± SD)**			
	TC	**TG**	**HDL**	**LDL**
0 hour before triton	121.1 ± 27.9	81.0 ± 11.31	57.8 ± 11.2	46.6 ± 12.2
2 days after triton	391.2 ± 31.2	398.1 ± 51.7	61.7 ± 11.3	143.1 ± 22.8
Difference (%)	223	390	7	207
7 days after triton	379.8 ± 42.8	388.1 ± 58.5	59.8 ± 12.4	139.6 ± 14.9
Difference (%)	214	377	3	199
14 days after triton	283.4 ± 26.5	334.7 ± 11.4	59.3 ± 12.1	129.7 ± 14.6
Difference (%)	134	312	3	178
21 days after triton	228.7 ± 12.1	327.4 ± 16.7	59.1 ± 11.3	122.7 ± 16.4
Difference (%)	89	303	2	163

Effect of standard drug (Atorvastain) on lipid profile status in triton induced hypercholesterolemia albino rats

In this research work, the Group II experimental data of mean plasma levels of albino rats in 2 days after administration of triton WR-1339 (400 mg/kg b. wt) and in 7 days, 14 days and 21 days after treatment with Atorvastain (10 mg/kg b.wt) as standard drug are shown in Table 5. After administration of triton WR-1339 (400 mg/kg b. wt) obviously increased in levels of TC by 181 %, TG by 184 % and LDL by 308 % and decreased in HDL level by 7 % within 2 days. Treatment with Atorvastain (10 mg/kg b.wt) significantly decreased in levels of 59 % TC, 65 % TG and 42 % LDL and increased in 21 % HDL level of cholesterol within 21 days.

Table 5. Mean Plasma Lipid Levels of Group II Receiving Atorvastatin 10 mg/kg b.wt dose as Standard Drug Group for Water Soluble Chitosan after 2 days, 7 days, 14 days and 21 days

Mean Plasma Lipid Levels (mg/dL)	**Lipid Profile (mean ± SD)**			
	TC	**TG**	**HDL**	**LDL**
0 hour before triton	124.1 ± 28.1	104.8 ± 21.8	70.8 ± 17.1	33 ± 11.2
2 days after triton	348.2 ± 11.5	298.1 ± 41.3	65.9 ± 14.5	134.6 ± 23.7
Difference (%)	181	184	7	308
7 days after triton + Atorvastatin	276.8 ± 11.9	213.4 ± 34.1	67.1 ± 11.6	118.6 ± 23.4
Difference (%)	21	28	2	12
14 days after triton + Atorvastatin	211.7 ± 19.4	181.4 ± 31.8	73.7 ± 14.8	107.1 ± 17.9
Difference (%)	39	39	12	20
21 days after triton + Atorvastatin	144.1 ± 11.2	103.8 ± 6.4	79.4 ± 4.8	48.1 ± 14.1
Difference (%)	59	65	21	42

Effect of water soluble chitosan on lipid profile status in triton induced hypercholesterolemia albino rats

The test Group III, 2 days after administration of triton WR-1339 (400 mg/kg body weight) significantly increased in levels of TC by 169%, TG by 249 % and LDL by 297 % and decreased in HDL level by 8 %. Treatment with WS-chitosan at a dose of (400 mg/kg body weight) not significantly decreased in levels of TC by 64 %, TG by 77 % and LDL by 49 % and increased in HDL by 14 % at 21 days. These data are shown in Table 6. In Group IV, 2 days after administration of triton WR-1339 (400 mg/kg) significantly increased in levels of TC by 188 %, TG by 203 % and LDL by 349 % and decreased in level of HDL by 14 %. Treatment with WS-chitosan samples (500 mg/kg) evidently decreased in levels of TC by 67%, TG by 71% and LDL by 40% and increased in level of HDL by 24 % in 21 days (Table 7). In Group V, 2 days after administration of triton WR-1339 (400 mg/kg) significantly increased in levels of TC by 195 %, TG by 258 % and LDL by 351 % and increased in level of HDL by 9 %. After treatment with WS-chitosan (600 mg/kg b.wt) evidently decreased in levels of TC by 59 %, TG by 62 % and LDL by 40 % but increased in HDL level by 26 % at 21 days shown in Table 8.

According to the data of Groups III, IV and V, the Group V (WS-chitosan 600 mg/kg) showed the highest antilipidemic activity than others.

Table 6. Mean Plasma Lipid Levels of Group III Receiving Water Soluble Chitosan (400 mg/kg b.wt) after 2 days, 7 days, 14 days and 21 days

Mean Plasma Lipid Levels (mg/dL)	**Lipid Profile (mean ± SD)**			
	TC	**TG**	**HDL**	**LDL**
0 hour before triton	123.7 ± 30.9	114 ± 11.3	75.8 ± 20.8	25.5 ± 15.8
2 days after triton	332.3 ± 12.7	397.8 ± 27.1	69.9 ± 6.7	101.3 ± 11.4
Difference (%)	169	249	8	297
7 days after triton + WS-chitosan (400 mg/kg)	252.1 ± 11.7	181.7 ± 19.6	71.9 ± 21.3	85.6 ± 19.4
Difference (%)	32	54	3	15
14 days after triton + WS-chitosan (400 mg/kg)	209.6 ± 28.3	179.8 ± 31.4	78.1 ± 11.7	73.8 ± 17.6
Difference (%)	37	55	12	27
21 days after triton + WS-chitosan (400 mg/kg)	118.3 ± 13.6	89.4 ± 14.8	78.8 ± 21.3	62.1 ± 31.7
Difference (%)	64	77	14	39

Table 7. Mean Plasma Lipid Levels of Group IV Receiving Water Soluble Chitosan (500 mg/kg b.wt) after 2 days, 7 days, 14 days and 21 days

Mean Plasma Lipid Levels (mg/dL)	**Lipid Profile (mean ± SD)**			
	TC	**TG**	**HDL**	**LDL**
0 hour before triton	115 ± 17.1	94 ± 21.1	77 ± 15.9	19.3 ± 11.1
2 days after triton	331.6 ± 12.8	284.8 ± 71.9	66.1 ± 11.3	86.8 ± 15.4
Difference (%)	188	203	14	349
7 days after triton + WS-chitosan (500 mg/kg)	208.9 ± 17.6	174.3 ± 23.4	70.6 ± 11.2	68.9 ± 7.3
Difference (%)	37	39	7	21
14 days after triton + WS-chitosan (500 mg/kg)	178.6 ± 24.6	87.4 ± 31.7	75.8 ± 11.4	63.1 ± 6.6
Difference (%)	46	69	15	27
21 days after triton + WS-chitosan (500 mg/kg)	109.8 ± 11.7	83.4 ± 6.1	81.9 ± 23.1	51.8 ± 16.4
Difference (%)	67	71	24	40

Table 8. Mean Plasma Lipid Levels of Group V Receiving Water Soluble Chitosan (600 mg/kg b.wt) after 2 days, 7 days, 14 days and 21 days

Mean Plasma Lipid Levels (mg/dL)	Lipid Profile (mean ± SD)			
	TC	TG	HDL	LDL
0 hour before triton	107.0 ± 31.1	75.4 ± 31.5	63.5 ± 15.2	27.7 ± 6.1
2 days after triton	315.6 ± 27.8	288.9 ± 34.1	69.4 ± 14.4	124.9 ± 13.8
Difference (%)	195	258	9	351
7 days after triton + WS-chitosan (600 mg/kg)	208.9 ± 31.6	154.3 ± 11.3	72.8 ± 6.5	93.7 ± 21.6
Difference (%)	34	47	5	25
14 days after triton + WS-chitosan (600 mg/kg)	183.1 ± 11.7	153.1 ± 16.4	81.4 ± 21.2	81.6 ± 24.6
Difference (%)	42	47	17	35
21 days after triton + WS-chitosan (600 mg/kg)	128.5 ± 14.6	110.6 ± 20.8	87.7 ± 11.7	74.9 ± 19.6
Difference (%)	59	62	26	40

Conclusion

From the assessment of the antimicrobial activity, the water soluble chitosan was more effective on gram positive bacteria namely *Bacillus subtilis, Bacillus pumilus* and *Staphylococcus aureus* than others. By the investigation of the antitumor activity, the minimum inhibitory concentration of WS-chitosan was found to be 3.90 µg/disc. Antioxidant activity test was assessed by DPPH assay revealed by mild radical scavenging activity of water soluble chitosan. According to the investigation of antioxidant activity, the value of the IC_{50} of the water soluble chitosan was 4.20 µg/mL was evidently comparable with standard ascorbic acid (IC_{50} = 1.43 µg/mL). From acute toxicity test, it

was observed that the water soluble chitosan was free from toxic effect in the range of 1-5 g/kg body weight of albino mice. The concentration (600 mg/kg) of WS-chitosan showed the highest activity and more weight loss effect on female albino mice than male albino mice. The hypocholesterolemic or antilipidemic activity of the water soluble chitosan was determined on albino rat model using triton WR-1339 injection method. In this study, it indicated that water soluble chitosan has the ability to reduce "bad" cholesterol (TC, TG and LDL) and it also could significantly promote the "good" blood cholesterol (HDL) level especially the concentration of WS-chitosan at (600 mg/kg body weight) within 21 days. Thus, water soluble chitosan can be suitable for some applications particularly in health care products, nutrition, pharmacology and cosmetic additive process.

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