ACHATINA FULICA EXOSKELETON DERIVED CHITOSAN ATTENUATES LIVER AND KIDNEY TOXICITIES IN DEXAMETHASONE INDUCED HYPERTENSIVE MALE WISTAR ALBINO RATS

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ABSTRACT

Chitosan, a natural mucopolysacharide obtained from chitin of animal exoskeletons have been suggested to play vital medical roles. Its effects have been investigated in patients with chronic renal failure undergoing long term stable haemodialysis treatment. However antioxidative and antihepatotoxic effects might not have been reported, hence this study was investigated to monitor the anti-oxidative and anti-toxicity effects of small snails (*Achatina fulica*) exoskeleton derived chitosan in the liver, kidney and plasma of dexamethasone induced hypertensive rats. The animals were acclimatized for two weeks before administration of the drug. The animals were divided into four groups of five rats each with various treatments and administrations which lasted for three weeks. After which the animals were anesthetized, dissected and [catalase, superoxide dismutase (SOD), alkaline phosphatase (ALP), aspartate transaminase (AST) and alkaline transaminase (ALT)] activities; reduced glutathione, cholesterol and malondialdehyde (MDA) status were evaluated the plasma, kidney and liver homogenates. The results obtained showed that dexamethasone actually induced significant stress in both organs of the hypertensive rats with elevation of some enzyme activities which is indicative of organ toxicity; cholesterol and MDA a marker of lipid peroxidation with reduced GSH and catalase while chitosan manifested its liver and kidney anti-toxicity and anti-oxidative potentials by assuaging the enzyme activities and metabolites in plasma and the organs.

Keywords: Organ toxicity, oxidative stress, hypertension, dexamethasone, chitosan, antioxidant.

INTRODUCTION

Chitosan, a linear copolymer composed by β-(1→4)-2-acetamido-D-glucosamine (GlcNAc), produced from chitin through the N-deacetylation with varied degree of acetylation, is nontoxic and biodegradable. Composed by GlcNAc, chitosan and chitin have similar molecular structures; the differences are that chitin is 50 to 100% acetylated while chitosan is 0 to 50% acetylated (Yoon *et al.*, 2001; Johnsen *et al.*, 2010; El-Sherbiny, 2011). Chitosan and chitin can be converted into highly water-soluble oligosaccharides by showing anti-tumor, anti-microbial and immunopotentiating biological activities (Zakrzewska *et al.*, 2005; Son *et al.*, 2003; Cheng et al., 2006). They exhibit broad applications in biomedical, pharmaceutical, agricultural and biotechnological fields (Casal *et al.*, 2006; Samdancioglu *et al.*, 2006; Ishihara et al., 2006).

Chitosan is shown to have superior characteristics and especially flexibility in its use. The use of dietary fiber has been attractive because of the reduction in the energy density of the diet (Beereboom, 1979), an improvement in bowel habits (Cummings *et al.*, 1976) and the prevention of colon cancer (Burkitt, 1971). Chitosan, a polyglucosamine derived from chitin,

is a cellulose-like polymer located mainly in the exoskeletons of arthropods, such as crabs, shrimps, lobsters and insects (Razdan and Pettersson, 1994).

It can be defined both chemically and physiologically as a dietary fibre since it is a polysaccharide, which cannot be digested by digestive enzymes of humans (Razdan and Pettersson, 1996). Moreover, it is the only abundant polysaccharide derived from animals, and its cationic characteristics are different from other dietary fibers (Muzzarelli, 1996). It is natural and nontoxic, and growing evidence indicate that it exhibits a marked hypolipidemic activity that would reduce the risk of cardiovascular diseases (Zhou *et al.*, 2006). It has exhibited a potent hypertensive activity in rats (Simunek and Bartonova, 2005 and Liu *et al.*, 2008) and humans (Guerciolini *et al.*, 2001; Maezaki *et al.*, 1993). Previous studies have reported that chitosan reduced the risk in animals (Chiang *et al.*, 2000; Yao and Chiang, 2002, 2006). Chitosan [poly- (B-1-4)-D-glucosamine] is prepared by the deacetylation of chitin using concentrated alkali (Krisana *et al.,* 2004; Shepherd *et al.*, 1997). It has been reported to possess immunological (Nishimura *et al.*, 1984; Mori *et al.*, 1997) antibacterial (Tokura *et al.*, 1997; Tanigawa *et al.*, 1992) and wound healing activities (Okamoto *et al.*, 1993; Kweon *et al.,* 2003; Khnor and Lim, 2003).

Chitosan, a polyglucosamine derived from chitin, is a cellulose-like polymer located mainly in the exoskeletons of arthropods, such as crabs, shrimps, lobsters and insects (Razdan and Pettersson, 1994). It can be defined both chemically and physiologically as a dietary fibre since it is a polysaccharide, which cannot be digested by digestive enzymes of humans (Razdan and Pettersson, 1996). Moreover, it is the only abundant polysaccharide derived from animals, and its cationic characteristics are different from other dietary fibers (Muzzarelli, 1996). It is natural and nontoxic, and growing evidence indicate that it exhibits a marked hypolipidemic activity that would reduce the risk of cardiovascular diseases (Zhou *et al.*, 2006). It has exhibited a potent hypocholesterolemic activity in rats (Simunek and Bartonova, 2005 and Liu *et al.*, 2008) and humans (Guerciolini *et al.*, 2001; Maezaki *et al.*, 1993). Previous study have reported that chitosan reduced the concentration of plasma cholesterol in animals (Chiang *et al.*, 2000; Yao and Chiang, 2002, 2006a,b) and type II diabetes patients in combination with hypercholesterolemia (Tai *et al.*, 2000 and Yao *et al.*, 2008). Increased fecal cholesterol accompanied with or without bile acid excretion by interfering intestinal micelle formation was proposed to be the mechanisms responsible for the hypocholesterolemic properties (Gallaher *et al.*, 2000; Yao and Chiang, 2006). Maezaki *et al.* (1993) was the first to report the hypocholesterolemic effect of chitosan in humans and found that chitosan effectively decreased plasma lipid level and had no side effect. Han *et al.,* (1999) has reported that supplementation of chitosan can reduce high fat diet-induced lipidemia by its antilipidemic property. The free radical quenching property of this marine polysaccharide has also been studied in detail (Xing *et al.,* 2005). Reports by Filipovic-Grcic *et al.,* (2001) indicate the membrane stabilizing property of chitosan. Since chitosan can wrap solid particles in liquids to bring them together and agglomerate, it is effectively utilized for this controlled release of drug (Krisana *et al.*, 2004). Dissolution properties and bioavailability of poorly soluble drugs can be improved by grinding them with chitosan. Muzzarelli (1996) has reported that supplementation of chitosan can prevent experimentally induced diabetic complications by its antioxidant and membrane stabilizing properties. Though the beneficial effects of chitosan have been extensively studied, the antihepatotoxic and possibly antinephrotoxic potentials of chitosan on liver and kidney toxicities in dexamethasone induced hypertension has not yet been explored. Therefore, this study was aimed at inducing hypertension with the dexamethazone on the rats, treat same

with chitosan and then observe the consequences, remedy and or otherwise on the plasma, liver and kidney.

MATERIALS AND METHODS

Chitosan was extracted from shells of *Achatina fulica* obtained locally from Ado-Ekiti, Nigeria. Wistar albino rats were obtained from animal breeder around Federal University of Technology Akure, Ondo State, Nigeria. They were housed in ventilated cages in the Animal House of Biochemistry Department, Ekiti State University, Ado-Ekiti, Nigeria.

Extraction of chitin and chitosan from *Achatina Fulica* exoskeleton:

SHELL

(Washed and dried)

↓

GRINDING AND SIEVING

↓

DEPROTEINIZATION

(25g of powdered sample was dissolved in 3.5% of NaOH) solid: solvent (1:10 w/v) for 2 hours at 65^0C

↓

WASHING

(The resulting sample was washed severally)

↓

DEMINERALIZATION

(The washed sample was dissolved in 1N HCl solution) solid: solvent (1:15 w/v) for 30 minutes at room temperature

↓

WASHING

(The resulting sample was washed severally)

↓

DECOLORATION

The extract was washed with acetone and bleached with 0.315% of NaOCl, solid: solvent (1:10 w/v) for 5 minutes at room temperature.

↓

WASHING AND DRYING

↓

CHITIN

↓

DEACETYLATION

100ml of 50% NaOH was added to the chitin and then boiled at 100^0C for 1hour on a hot plate.

(The resulting sample was washed severally)

↓

CHITOSAN

Figure 1: Flow chart for the production of Chitosan from *Achatina fulica*
(modified from Meyer, 1995).

Experimental Animals

20 male Wistar albino rats, 2 to 4 weeks old, weighing between 10-35 g were housed and kept in an iron cage and maintained under standard laboratory conditions with free access to rat pellets and water until weight gained between 75-100 g.

Induction of Hypertension in Experimental Animals with Dexamethasone:

Hypertension and oxidative stress on the organs were induced by oral administration of dexamethasone (1.67mg/kg body weight) dissolved in normal saline to the animals.

Experimental Design

Twenty male Wistar albino rats were housed in ventilated cages in the Animal House of Biochemistry Department, Ekiti State University, Ado-Ekiti, Nigeria. They were randomly assigned into four groups of five rats each which were acclimatized for two weeks before administration of the drug and treatments that lasted for three weeks. Standard pellet diet and water was provided *ad libitum* to the animals which were kept at optimum temperature with a 12 hour light/dark cycle during the period of study.

GROUP A: This group served as the positive control. The animals in this group were only given food and water.
GROUP B: The animals in this group were administered with aqueous extract of *Achatina fulica* derived chitosan every other day in addition to food and water.
GROUP C: This group served as the negative control. The animals in this group were administered with 0.5mL oral dose of dexamethasone (1.67 mg/kg body weight) every other day in addition to food and water.
GROUP D: The animals in this group were administered with 0.5 mL oral dose of dexamethasone (1.67 mg/kg body weight) as in group C but in addition followed with 0.5 mL oral administration of aqueous extract of *Achatina fulica* exoskeleton derived chitosan as in group B.

Preparations of plasma and tissues homogenates

Under ether anaesthesia, the animals were dissected and blood was collected from the heart using needle and syringe which was immediately transferred into an anti-coagulant bottle which was centrifuged to obtain the plasma. The 10% each of kidney and liver homogenates were prepared in 6.7mM potassium phosphate buffer, (pH 7.4) using the Teflon homogenizer. The homogenates were centrifuged at 10,000rpm for 10 minutes at 4^0C to obtain a clear supernatant which was stored at 8^0C and used for measurement of [catalase, superoxide dismutase (SOD), alkaline phosphatase (ALP), aspartate transaminase (AST) and alkaline transaminase (ALT)] activities; reduced glutathione, cholesterol and malondialdehyde (MDA) status in the plasma and liver as well as urea and creatinine concentrations analyzed in the plasma and kidney homogenates.

Standard Randox kits were used to determine Cholesterol, total protein, alkaline phosphatase (ALP), aspartate transaminase (AST) and Alkaline Transaminase (ALT). The methods of Sinha (1972), Misra and Fridovich (1972), Jollow *et al*. (1974) were employed for the determinations of (catalase, superoxide dismutase) activities, reduced glutathione respectively

while modified method of Varshney and Kale (1990) was used to determine malondialdehyde (MDA),

Statistical Analysis

The triplicate results were obtained and expressed as mean±SD (standard deviation) which was used to compare the changes occurring due to the effects of the extract and the drug in the liver, kidney and the plasma to prove the anti-toxicity and anti-oxidative effects of *Achatina fulica e*xoskeleton derived chitosan on toxicity and oxidative stress in dexamethasone induced hypertensive male Wistar albino rats.

RESULTS AND DISCUSSION

The liver and kidney are central organs for many physiological and biochemical processes necessary for maintenance of life (Souba and Wilmore, 1983). Morphological alterations that occur in these organs affect many metabolic processes in the organism. Dexamethasone is known to raise systolic blood pressure and to decrease thymus weight and the rate of body-weight gain (Zhang et al., 2004).

Peroxide formation induced by hypertension result in the release of some enzymes by interacting with cellular structure and function; thus, the plasma activities of cellular enzymes such as transaminases (AST and ALT) and alkaline phosphatise will be affected. With the increase in cellular membrane permeability, intracellular fluid transfers into intercellular space, resulting in muscle and liver cell degeneration. In this study, it was observed that as a result of enzymes such as AST, ALT and ALP released into blood, the increase in the plasma activities of these enzymes was directly proportional to the degree of cellular damage (Sudhahar *et al.*, 2007). The increase in their activities was however decreased by chitosan. Tables 1-3 below present the results of liver, kidney and plasma AST, ALT and ALP activities in the controls and experimental groups.

Table 1.0: Specific activity of aspartate transaminase in selected tissues of hypertension induced rats treated with extract of *Achatina fulica* chitosan

	Specific activity (IU/mg protein)		
Groups	**Liver**	**Kidney**	**Plasma**
A	123.00 ±5.98	82.50±6.42	29.50±2.88
B	274.00±8.34	136.45±8.55	89.00±7.89
C	420.00±4.86	215.50±5.15	123.00± 4.23
D	280.00±6.86	93.28±4.14	47.00±2.78

Each value is a mean of triplicate results ± SD.

There were significant increases in the of liver, kidney and plasma of AST, ALT and ALP activities of hypertensive-induced rats as compared to normal control rats . The present finding are in agreement with those obtained by Ahmed *et al.* (1987) who found that hypertension state significantly stimulate ALT, AST and ALP activity mostly in the plasma and little in the liver. Plasma ALT activity highly decreased in hypertensive-induced rat treated with ikoto chitosan, compared with hypertensive induced rats.

Table 2.0: Specific activity of Alanine Transaminase in selected tissues of hypertension induced rats treated with aqueous extract of *Achatina fulica* chitosan

	Specific activity (IU/mg protein)		
Groups	**Liver**	**Kidney**	**Plasma**
A	115.20 ±3.48	3.62 ±0.22	67.00 ±3.67
B	21.00 ±2.98	59.21±0.42	43.20 ±1.43
C	169.80 ±5.60	17.80 ±1.04	123.00 ±4.33
D	95.80 ±4.98	4.20 ±0.52	93.80 ±2.64

Each value is a mean of triplicate results ± SD.

Moreover, plasma AST and ALP activity are significantly decreased in all treated groups as compared to hypertensive-induced negative control rat. The present findings also agree with those obtained by Ahmed *et al.* (1987) that found that hypertensive state highly stimulate ALT, AST and ALP activity mostly in the plasma and slightly in other tissues.

Table 3.0: Specific activity of Alkaline Phosphatase in selected tissues of hypertension induced rats treated with aqueous extract of *Achatina fulica* chitosan

	Specific activity (IU/mg protein)		
Groups	**Liver**	**Kidney**	**Plasma**
A	49.68±3.98	549.24±5.12	35.88±2.88
B	112.00±6.34	281.52±3.18	114.50 ±7.89
C	212.00± 7.86	389.16±4.84	154.00 ± 4.23
D	129.00± 6.86	212.52±2.86	102.00 ± 2.78

Each value is a mean of triplicate results ± SD.

In the present study, dexamethasone induction enhances the cholesterol synthesis significantly in the liver and the plasma as seen in Table 4, this result however corroborate the observation of (Shinnick *et al.*, 1990) though the effect is not all that significant in the kidney. The ethanolic extract however caused significant reversal in the cholesterol synthesis bringing the values almost to the control level. Earlier researchers have also reported that chitosan significantly lowering plasma and liver cholesterol in dexamethasone -induced hypertensive rats (Yao *et al.*, 2008; Liu *et al.*, 2008; Ormrod *et al.*, 1998). The strong positive charge carried by the chitosan molecule (amino groups) causes it to bind negatively charged substrates such as lipids. Chitosan binds fat in the intestine, blocking absorption, and has been shown to lower blood cholesterol in animals and humans (Ormrod *et al.*, 1998 and Gallaher *et al.*, 2000). Reduction of fatty acid and bile acid will lead to less absorption of fat from the diets (Gallaher *et al.*, 2000), and the reduction of endogenetic cholesterol because of the interruption of enterohepatic bile acid circulation (Razdan and Pettersson, 1996), will influence cholesterol metabolism. Chitosan is soluble in the acidic conditions of the stomach and forms a gel when the molecular weight is high. When fat and chitosan in the diets are eaten together, the viscous chitosan will entrap the fat droplet in the stomach. In the small intestine, which is at neutral pH, chitosan forms a precipitate and prevents the digestion of fat (Zhou *et al.*, 2006). Tsujikawa *et al.*, (2003) and Kanauchi *et al.*, (1994) speculated that gastric acid–soluble chitosan mixes with dietary fat in the stomach, with the emulsifying process effectively mediated by ascorbic acid.

Table 4.0: Total cholesterol concentration in selected tissues of hypertension induced rats treated with aqueous extract of *Achatina fulica* chitosan

	mMol/dL		
Groups	**Liver**	**Kidney**	**Plasma**
A	16.40± 2.10	11.97±4.07	60.48± 6.34
B	10.08± 1.23	25.20±4.07	71.19± 4.23
C	90.09± 6.06	16.38±4.07	101.43±6.98
D	30.03± 3.07	8.82±4.07	68.04±4.07

Each value is a mean of triplicate results ± SD.

The observed hypolipidaemic effect may be due to decreased cholesterogenesis and fatty acid synthesis. Significant lowering of total cholesterol is a very desirable biochemical state for prevention of hypertension and ischaemic conditions (Jouad *et al.*, 2003; Daisy *et al.*, 2009). These studies confirmed the present study which indicated that, small snail (*Achatina fulica*) chitosan,has more lowering effect of lipid profile . In fact it may prevent the increase in the factors causing coronary heart diseases (CHD) and cardiovascular diseases (CVD), therefore it may prevent of atherosclerosis.

Table 5.0 showed reduced glutathione (GSH) concentration in liver, kidney and plasma. A significant decrease in the amount of glutathione may result in the destruction of membrane integrity (Kempaiah and Srinivasan, 2005 and Tauseef *et al.*, 2007). In this study, the significant decrease in the liver and plasma reduced glutathione concentration of hypertensive group may cause damage to hepatocytes and erythrocyte membranes integrity while the observed increase in the amount of GSH in the chitosan fed group effectively protect membrane integrity. It is worthy to note that the concentration of reduced glutathione in the kidney is low as seen in Table 5.0 and a reverse in the concentrations of reduced glutathione in liver and plasma was observed in the kidney.

Table 5.0: Reduced glutathione (GSH) in selected tissues of hypertension induced rats treated with aqueous extract of *Achatina fulica* chitosan.

	(ug/ml)		
Groups	**Liver**	**Kidney**	**Plasma**
A	0.611 ±0.13	0.11±0.01	0.641±0.22
B	0.509 ±0.15	0.04±0.01	0.530 ±0.04
C	0.343 ±0.10	0.25±0.01	0.427 ±0.06
D	0.555±0.12	0.06±0.01	0.522 ±0.05

Each value is a mean of triplicate results ± SD.

A significant increase in the amount of MDA concentration of detamethazone induced hypertensive rats in Table 6.0 may result in the destruction of membrane integrity (Kempaiah and Srinivasan, 2005; Tauseef *et al.*, 2007).

Table 6.0: Malondialdehyde concentration in selected tissues of hypertension induced rats treated with aqueous extract of *Achatina fulica* chitosan)

	(Units/ mg) x 10^{-3}		
Groups	**Liver**	**Kidney**	**Plasma**
A	2.87± 0.20	1.16±0.10	6.77±0.40
B	2.68±0.12	1.12±0.20	5.49±0.50
C	7.30±0.14	3.58±0.20	98.20±0.70
D	1.78±0.22	1.34±0.20	10.93± 0.60

Each value is a mean of triplicate results ± SD.

In this study however, the significant increase in the liver, kidney and plasma malondialdehyde levels of hypertensive group may cause damage to the integrity of the hepatocytes, nephrocytes and erythrocyte membranes as a result of lipid peroxidation. On the other hand, the observed decrease in MDA liver, kidney and plasma in the chitosan fed groups effectively protect membrane integrity.

Hypertensive-induced rat showed significant increase in plasma and liver malondialdehyde (MDA) level when compared to control groups (A and B) Table 6.0. MDA significantly increased in hypertensive group C Table 6.0. The observations in this study however agree with the results of Kempaiah and Srinivasan (2004 and 2005) in their work.

CONCLUSION AND RECOMMENDATION

A lot of synthetic drugs from the market used to cure major diseases appeared to cause devastating side effects on some organs which can lead to induction of hepatotoxicity and the likes. Chitosan is one of the most abundant natural aminopolysaccharides with variety of applications; Its negative side effects have not been reported while its immunological, antibacterial, haemostatic, fungistatic, antitumoral, anticholesteremic properties and wide pharmaceutical applications have widely been reported. Hence, the present study revealed the medicinal importance of small snail chitosan especially in the liver, kidney and plasma of dexamethasone induced hypertensive rats to reverse the adverse effects caused by the drug and protective the tissues. However, further safety studies and investigations may be embarked on the structural elucidation of the compounds with the aim of identifying the bioactive compounds.

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