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RESEARCH ARTICLE

EXPERIMENTALLY-INDUCED HYPERTENSION IN WISTAR RATS: THE ROLE OF ACHATINA FULICA DERIVED CHITOSAN

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ABSTRACT

The scourge of hypertension has led to the continued search for alternative drug therapy that will be effective, affordable and devoid of side effects. Chitosan has been demonstrated to have the ability to scavenge free radicals generated as products of oxidative stress in various diseases.
This study investigates the effects of *Achatina fulica* derived Chitosan on the morphology of the kidney, cardiac muscles, liver and spleen in experimentally-induced hypertension in Wistar rats.
Twenty male Wistar rats were assigned into four groups of five rats each. Group A served as the control, group B received aqueous extract of *Achatina fulica* derived chitosan, Group C rats were given dexamethasone (1.67 mg/kg b. w.) while Group D rats same dose of dexamethasone in addition to 0.5 mL of aqueous extract of *Achatina fulica* derived chitosan. Treatments were by gavage every other dayand lasted forthree weeks.
Under ether anaesthesia, the animals were dissected; the liver, kidney, spleen and the heart were excised, fixed in 10% NBF and processed by paraffin wax embedding method. Tissue sections were produced on a rotary microtome and stained with haematoxylin and eosin.
The stained sections were examined and photographed with an Axioscope A1 microscope version 4.83 (Carl Zeiss, Germany).
Results from our study showed evidence of disruption in the microanatomy of tissues in the dexamethasone treated group of rats while Chitosan played a protective role in preventing these effects. This may be due to the ability of Chitosan to mop-up products of oxidative stress.

INTRODUCTION

Non-communicable diseases such as hypertension, a chronic condition of elevated blood pressure and risk factor for cardiovascular diseases have become a cause for global concern.[1,2] About a billion people worldwide are affected by hypertension otherwise referred to as the “silent killer” as it has no distinct sign and symptom at the initial stage.[3] Africa may be most the affected continent in the world as 46% of adults aged 25 years and above are affected; with the skyrocketing population of Nigeria and the shift to Western lifestyle, the impact of the disease may be alarming and on. [4] A study in a Nigerian population also suggests that one in three persons have a risk of developing hypertension.[5] Globally, hypertension alongside other non-communicable diseases worsens poverty levels and impairs meaningful development by reducing available national income.[2] The economic burden due to hypertension and the cardiovascular complications such as cerebro-vascular accidents, heart failure, and renal failure is very high.[6] With the failure of the Millennium Development Goals (MDGs) in Nigeria and the threat of increase in burden of hypertension; there is need for reinforcement of efforts for the success of the Sustainable Development Goals (SGDs).[7] In an effort to bring an end to poverty and ensure good health and wellbeing of the populace, there is need for proactive measures in finding alternative, efficient and cost-effective measures to reduce the economic impact, morbidity and mortality due to hypertension’.

The side effects associated with conventional therapies for the management of hypertension include orthostatic hypotension, increased plasma cholesterol, reduced glucose tolerance and sexual dysfunction among others. These have led to the intensification of the search for alternative therapies devoid of side effects. Various natural products have been found to have efficacious antihypertensive potential in this regard.

Chitosan is a naturally occurring bio-polymer present as a major component in the mycelial and sporangiophore walls of fungi and exoskeleton of insects, crustaceans and molluscs such as the giant African land snail, *Achatina fulica.*[8] Chitosan is a fibre-like cellulose but unlike plant fibre, it possesses unique properties such as positive ionic charge, which gives it the ability to chemically bind with negatively charged fats, lipids and bile acids. [9] According to Knorr, [10] the shell of molluscs generally consists of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin (from which chitosan is extracted).

Asides from its application in waste water treatment, cosmetics, biotechnology, agriculture and the food industry; Chitosan has shown promising results in its use as a bioactive compound. Studies have shown its efficacy as an anti-microbial, anti-cholesterol and anti-tumour agent.[11] Chitosan has been approved as a food additive in Korea and Japan since 1995, respectively; higher antibacterial activity of chitosan at lower pH suggests that addition of chitosan to acidic foods will enhance its effectiveness as a natural preservative. [12] It has been demonstrated to produce a stable heparin-chitosan complex which induces re-epithelization of full thickness wounds in human skin. [13] Also, it has been found to be effective in the management skin burns and blood cholesterol, inhibition of dental plaques and applicable as a clotting agent; and in the construction of contact lenses and dental sutures. Oseni *et al*. [14] demonstrated the effectiveness of giant snail chitosan in reversing hepatic and renal toxicities in experimentally-induced hypertension. Oxidative stress has been implicated in the pathophysiology of cardiovascular disease. [15] Asides from the absence of toxicity, Chitosan has been found to have a high free radical scavenging property. [16] There is need for research on cheaper alternative remedies which are effective for the management of hypertension; asides from the threatening impact of the disease on the population, the adverse effects associated with conventional therapies and problem of adherence to treatment contributes to morbidity and mortality.

In this research, the effect of *Achantina fulica* Chitosan on the histopathology of the liver, kidney spleen and cardiac muscle in dexamethasone-induced hypertension in rats were studied.

MATERIALS AND METHODS

Preparation of extract

Shells of Giant African snails, *Achatina fulica* obtained from a local market in Ado-Ekiti, Nigeria were washed, dried, grinded and sieved. As adapted from the method of No and Meyers, [17] chitin was isolated by deproteinization, demineralization and decolorization. This was followed by conversion of chitin to chitosan by deacetylation with 50% sodium hydroxide solution at 121°C. The extract was then washed, dried and stored until use.

Experimental Animals

Twenty male Wistar albino rats weighing between 75-100 g were randomly selected from the colony raised in the Animal Holding of College of Medicine, Ekiti State University, Ado-Ekiti, Ekiti State. The rats were housed in polycarbonate cages with stainless steel wire lids, at a constant temperature of 22 ± 1°C, under a 12-hour light/dark cycle with free access to standard rat pellets and water. All experiments were carried out according to the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines [18]and in compliance with Institutional Animal Research Ethical guidelines.

Experimental Design

The rats were randomly assigned into four groups (A, B, C and D) of five rats each and acclimatized for a week before commencement of the experiment.

As described by Scoggins *et al*., [19] hypertension was induced in groups C and D by administration of dexamethasone (1.67 mg/kg b. w.) dissolved in normal saline; group A rats received the vehicle while Group B were given 0.5 mL aqueous extract of *Achatina fulica* derived chitosan every other day in addition to food and water. Group C were given the same dose of dexamethasone every other day while Group D rats received dexamethasone in addition to 0.5 mL of aqueous extract of *Achatina fulica* exoskeleton derived chitosan. All treatments were by gavage and lasted for three weeks.

At the end of the experimental period, under ether anaesthesia, the animals were dissected; the liver, kidney, spleen and the heart were excised, fixed in 10% NBF and processed by paraffin wax embedding method. Sections of 4-5 µm thickness were produced on a rotary microtome and stained with haematoxylin and eosin for demonstration of general architecture.

The stained sections were examined under an Axioscope A1 microscope version 4.83 (Carl Zeiss, Germany). Digital photomicrographs of the tissue sections were taken at various magnifications.

RESULTS

Histology of the kidney

As seen in Figure 1A and B, the control group of rats and those treated with Chitosan alone demonstrated similar features of an essentially normal kidney. Evidence of distortion in the histology of the kidney is seen in the dexamethasone treated group of rats; some tubules are irregular with accumulation of eosinophilic secretions (Figure 1C). The dexamethasone + Chitosan group (Group D) shows comparable features with the control.

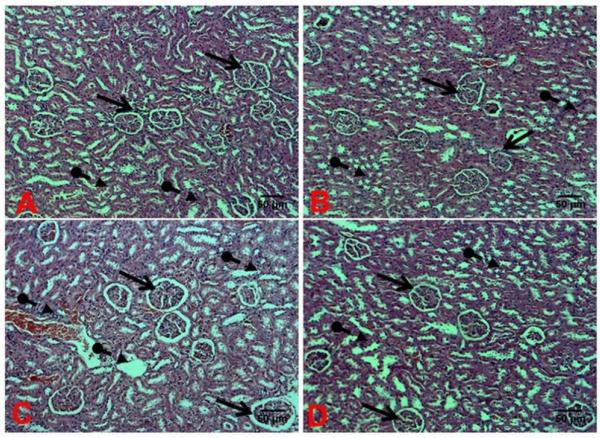

Figure 1 Kidney section of experimental rats (H & E). Note the normal looking kidney architecture with glomerulus (arrow) and tubules (dashed arrowhead) in groups A, B and D; Group C features splitting of some glomeruli, increased Bowman's space and evidence of tubular degeneration in few of the tubules.

Histology of the cardiac muscle

In figure 2, normal untreated rats showed normal cardiac fibres with regular striations and continuity with other myofibrils (figure 2A). Normal cardiac muscle bundles are seen in normal rats treated with Chitosan (figure 2B). Figure 2C shows the histopathological findings of dexamethasone treated rats with splitting of cardiac muscle fibre, inflammatory cells and few areas of necrosis. Chitosan treated group revealed normal cardiac muscle fibres with mild distortion in pattern of fibres (figure 2D).

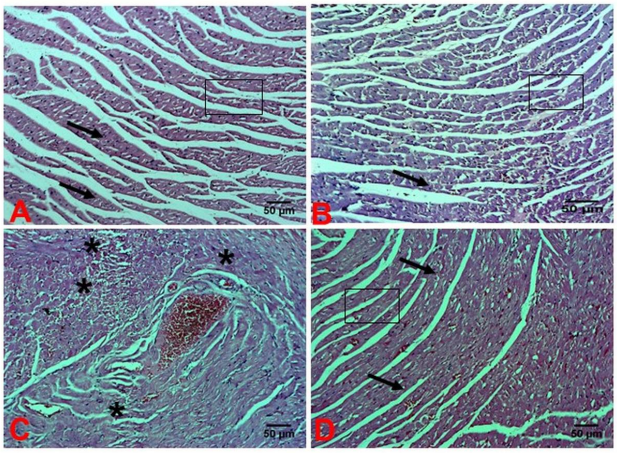

Figure 2 Cardiac musclesection of experimental rats (H & E). Note the muscle fibres with centrally located nuclei (arrows) and cylindrical branching of cardiac myocytes (box) in groups A, B and D; while group C shows irregular pattern of fibres (asterisk) and macrophage infiltration.

Histology of the Liver

Figure 3 features a normal looking sinusoidal arrangement of hepatocytes and central vein in the control (figure 3A), Chitosan treated (figure 3B) and Chitosan + dexamethasone treated groups of rats (figure 3D); while dilation and engorgement of blood vessels are observed in figure 3C.

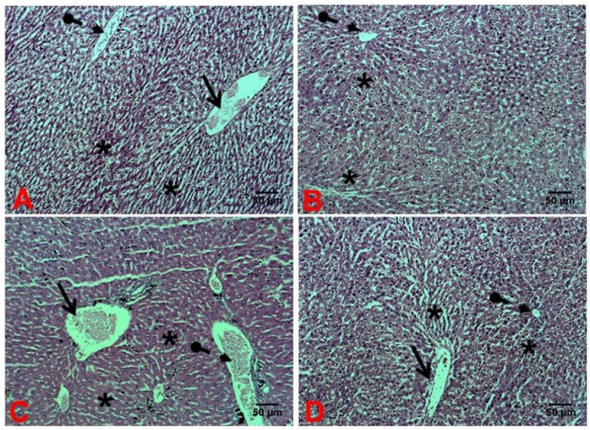

Figure 3 Liver section of experimental rats (H & E). A well preserved vascular relationship of the liver with the portal vein (arrows) and sheets of hepatocytes (asterisks) radiating from the central vein (dashed arrowhead) can be seen in groups A, B and D. Dilatation of the portal and central veins with disruption in the pattern of radiation of sheets of hepatocytes can be noted in Group C.

Histology of the spleen

A well-preserved relationship is seen in the sections of the spleen in the control and Chitosan only treated groups of rats (figure 4A and B). Mild distortion is observed in the splenic architecture, undefined boundary between the white and red pulps are observed in the dexamethasone group (figure 4C) while the Dexamethasone + Chitosan group displayed similar appearance with the control (figure 4D).

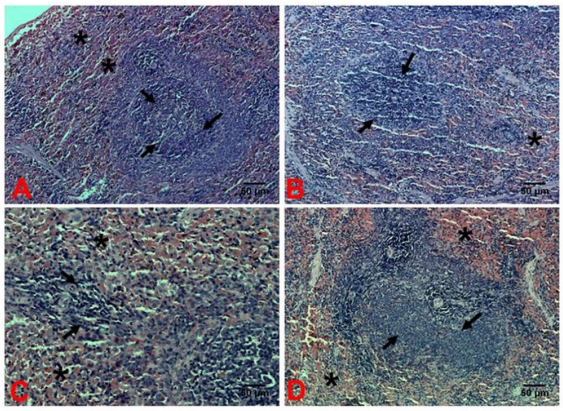

Figure 4 Spleen section of experimental rats (H & E). Groups A, B and D features well-formed white pulp (arrows) with variable red pulp (asterisks) while there is mild distortion in the appearances and delineation of red and white pulps in group C.

DISCUSSION

The pathophysiology of various forms of hypertension have been linked with formation of reactive oxygen species (ROS) which are upregulated in many animal models of hypertension. In hypertensive patients, increased ROS occurs as a result of oxidation of genomic and mitochondrial DNA.[20] Biomarkers of increased oxidative stress have been found to be increased in human hypertension.[20]

Dexamethasone is a synthetic glucocorticoid known for its potency in demonstrating glucocorticoid activity. Despite being efficacious as anti-inflammatory agents, prolonged use of glucocorticoids has been associated with various side effects including hypertension.[21] The hypertensive effect of dexamethasone has been demonstrated in many studies; [21] this is postulated to be via depleting the supply of Nitric oxide NO and subsequently causing vasoconstriction through the following mechanisms: upregulation of ROS level, reaction with Nitric oxide (NO), reduction in its bioavailability and inhibition of expression of reaction with NO synthase at the transcription level. [22]

Increased formation of ROS is known to cause tissue injury. [23] This is demonstrated in our study where administration of dexamethasone caused disruptive changes to the visceral organs of experimental animals. Antioxidants have been found to be potent for the reduction of dexamethasone-induced hypertension. [21] In this study, Chitosan had a protective role on the integrity of visceral organs as against the destructive effects found in dexamethasone treated rats.

Studies have shown conflicting evidence on alteration in renal structure and function preceding or following the progression of hypertension. Schwartz and Strong [24] proposed that the kidney is a target organ following development of hypertension. This is due to increase in renal vascular resistance. The resulting ischemia causes vascular lesions, glomerular and tubular changes. In this study, there were changes in the architecture of the kidney in dexamethasone treated rats. These changes were not evident in the dexamethasone + Chitosan group of rats. This shows the protective effects of Chitosan in preventing oxidative stress in Wistar rats. Findings from our study is also in concordance with that of Celsi *et al.* [25] who reported disruptive changes in

the glomerulus and subsequent development of hypertension in rats exposed to dexamethasone at different periods of gestation. They also found that exposure to elevated levels of glucocorticoids in utero resulted in impaired renal development and subsequently arterial hypertension in the offspring. [25]

Results from this study showed a distortion in the arrangement of cardiac muscle fibres with the presence of inflammatory cells. High doses of glucocorticoids have been reported to induce muscle atrophy in both humans and animals.[26] In many animal models, as an adaptive mechanism, hypertension is associated with atrophy and increased fibrosis of the cardiac muscle. [26] In skeletal muscles, glucocorticoids are known to down regulate protein synthesis while upregulating protein degradation.[28] In the heart, Gayan-Ramirez *et al*. [29] reported that glucocorticoids result in muscle mass atrophy by inhibiting IGF-1, a growth factor responsible for proliferation of myocytes; they also stimulate myostatin; a growth factor inhibiting proliferation of muscle mass [(30)]. The observed mild effects on the cardiac histology may be due to the limitation of the period of exposure to dexamethasone in this animal model.

Although the kidney and cardiac muscles are target organs in hypertension, the liver was investigated in this study because of evidence of the renin-angiotensin system (RAS) in the liver; this has been implicated in Non-Alcoholic Fatty Liver Disease which is also linked with hypertension.[31] There is an association between systemic and local RAS; [32] RAS have been implicated in the pathogenesis of hepatic fibrosis and studies have shown elevation of RAS in liver injury. [31] As the release of renin is mediated by the juxtaglomerular cells of the kidney, we hypothesized that an alteration of kidney morphology and function could affect the liver; however, in this study there was a mild dilatation of the portal tract and central veins in the dexamethasone treated rats. Evidence of disruption in the arrangement of hepatocytes was also observed while the other groups of rats had comparable features with the control.

Carnevale *et al.* [33] showed the involvement of placental growth factor (PIGF) from the splenic immune system in the pathogenesis of hypertension. In their study, the development of hypertension and targeted end organ damage was averted in PIGF deficient mice; this was due to the downregulation of T-cell infiltration by PIGF in the splenic reservoir. Asides its angiogenic effect, PIGF controls inflammation and transport of lymphocytes to target organs during hypertension; this is important for elevation of blood pressure and end organ damage.[34] In our study, the chitosan treated group of rats presented with similar features to the control group, however in the dexamethasone + chitosan group of rats, there was mild distortion around the white pulp. This could be due to the fact that T- cells are expressed in the marginal zone of the white pulp. Our results are in agreement with that of Rooman *et al.* [35] who reported that in both normal and transgenic mice, the thymus and spleen are organs most susceptible to glucocorticoid administration due to exaggerated rate of apoptosis in the cells. In another study, increased apoptosis in the follicular cells were associated with observed changes in the microstructure of dexamethasone treated rats.[36] In our study, co-administration of Chitosan with dexamethasone prevented these effects.

The anti-lipidemic effect of Chitosan and consequent reduction of risk of cardiovascular disease have been reported in both man and experimental animals. [37] Several studies have demonstrated the anti-oxidant effect of Chitosan. Araku *et al.*[15] showed that by its strong binding capacity, Chitosan down regulated the level of indoxyl sulfate, a pro oxidant; thus inhibiting production of ROS in haemodialysis patients.

In conclusion, to the best of our knowledge, this study is the first to demonstrate the protective role of Chitosan on the morphology of the kidney, cardiac muscles, liver and spleen in an animal model of hypertension. Treatment with *Achatina fulica* exoskeleton derived Chitosan prevented the dexamethasone induced damage to the histoarchitecture in these organs. This is thought to be due to the adsorptive abilities by which it binds to agents of oxidative stress in the gastrointestinal tract. There is need for further studies to identify the molecular mechanism involved in this anti-hypertensive effect and check the active ingredient under different routes of administration.

Figure legends

Figure 1. Kidney section of experimental rats (H & E). Note the normal looking kidney architecture with glomerulus (arrow) and tubules (dashed arrowhead) in groups A, B and D; Group C features splitting of some glomeruli, increased Bowman's space and evidence of tubular degeneration in few of the tubules.

Figure 2: Cardiac muscle section of experimental rats (H & E). Note the muscle fibres with centrally located nuclei (arrows) and cylindrical branching of cardiac myocytes (box) in groups A, B and D; while group C shows irregular pattern of fibres (asterisk) and macrophage infiltration.

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Figure 4: Spleen section of experimental rats (H & E). Groups A, B and D features well-formed white pulp (arrows) with variable red pulp (asterisks) while there is mild distortion in the appearances and delineation of red and white pulps in group C.

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