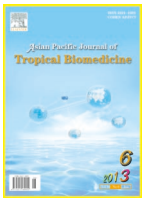
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:

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Modulatory effects of dietary inclusion of garlic (*Allium sativum*) on gentamycin-induced hepatotoxicity and oxidative stress in rats

Adedayo O Ademiluyi*, Ganiyu Oboh, Tosin R Owoloye, Oluwaseun J Agbebi

Functional Foods, Nutraceuticals and Phytomedicine Unit, Department of Biochemistry, Federal University of Technology, Akure, P.M.B. 704 Akure, 340001, Nigeria

PEER REVIEW

Peer reviewer

Akhere A. Omonkhua, PhD, Department of Medical Biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.
Tel: +2348053447581
E-mail: aaomonkhua@yahoo.com
akuekegbe.omonkhua@uniben.edu

Comments

This is a good study since it actually fills a gap in knowledge. The methodology is standard and presentation of results is adequate.
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ABSTRACT

Objective: To investigate the ameliorative effect of dietary inclusion of garlic (*Allium sativum*) on gentamycin-induced hepatotoxicity in rats. **Methods:** Adult male rats were randomly divided into four groups with six animals in each group. Groups 1 and 2 were fed basal diet while Groups 3 and 4 were fed diets containing 2% and 4% garlic respectively for 27 d prior to gentamycin administration. Hepatotoxicity was induced by the intraperitoneal administration of gentamycin (100 mg/kg body weight) for 3 d. The liver and plasma were studied for hepatotoxicity and antioxidant indices. **Results:** Gentamycin induces hepatic damage as revealed by significant ($P<0.05$) elevation of liver damage marker enzymes (aspartate transaminase and alanine aminotransferase) and reduction in plasma albumin level. Gentamycin also caused a significant ($P<0.05$) alteration in plasma and liver enzymatic (catalase, glutathione and super oxygen dehydrogenises) and non-enzymatic (glutathione and vitamin C) antioxidant indices with concomitant increase in the malondialdehyde content; however, there was a significant ($P<0.05$) restoration of the antioxidant status coupled with significant ($P<0.05$) decrease in the tissues' malondialdehyde content, following consumption of diets containing garlic. **Conclusions:** These results suggest that dietary inclusion of garlic powder could protect against gentamycin-induced hepatotoxicity, improve antioxidant status and modulate oxidative stress; a function attributed to their phenolic constituents.

KEYWORDS

Gentamycin, Hepatotoxicity, Oxidative stress, Garlic (*Allium sativum*), Phytochemicals

1. Introduction

The liver is a vital organ in the body, essential for life because it conducts vast array of biochemical and metabolic functions, including ridding the body of substances that would otherwise be injurious if allowed to accumulate, and excreting drug metabolites[1]. Hepatotoxicty or liver damage is the irregular functioning of the liver; with mitochondrial dysfunction reported as one of the major mechanism of drugs induce hepatotoxicity. By severely altering mitochondrial function in the liver, drugs can induce hepatic necrosis, causing cystolytic hepatitis, and can progress into liver failure[2]. When the liver is damaged, it may not be able to perform these functions optimally causing the accumulation of toxins in the body faster than the liver can process.

Garlic, is a commonly used food, and its medical properties have been well recognized since time immemorial. Garlic is a good source of compounds with a positive impact on human health and those compounds include sulphur-containing compounds and flavonoids, and findings have shown that garlic have profound beneficial health effects including antibacterial, anticarcinogenic, antinflammatory, hypolipidemic, hypoglycemic, antifungal,

*Corresponding author: AO Ademiluyi (Ph.D), Functional Foods, Nutraceuticals and Phytomedicine Unit,Department of Biochemistry, Federal University of Technology, Akure, P.M.B. 704 Akure, 340001, Nigeria.
Tel: +2348038044248
Fax: +2347063725898
E-mail: ademiluyidayo@yahoo.co.uk
Foundation Project: Supported by Federal University of Technology, Akure Staff Development Grant No. VCPU/URGC/46.

Article history:
Received 15 Mar 2013
Received in revised form 18 Mar, 2nd revised form 28 Mar, 13 Apr 2013
Accepted 10 May 2013
Available online 28 June 2013

and anti-atherosclerotic properties, and antioxidant activity[3].

A remarkable increase in the use of medicinal plant products has been observed in the past decade due to their active components and use in primary health care[4]. The human diet, which contains large number of natural compounds, is essential in protecting the body against the development of diseases, and *Allium sativum* (garlic, *A. sativum*) is one of the well known plants with remarkable anti-carcinogenic properties. Gentamycin is an aminoglycoside antibiotic derived from *Micromonospora purpurea* used in the treatment of many types of bacterial infections, particularly those caused by Gram-negative organisms[5]. Despite its wide clinical use, gentamycin have been reported to cause disturbing toxicity including nephrotoxicity and hepatotoxicity[6]. Noorani *et al*. reported that the administration of gentamycin for 7 d resulted in damage of liver structure with disarrangement of hepatic strands.

It has been shown that animal body had an effective mechanism to prevent the free radical induced tissue cell damage, this is accomplished by a set of endogenous antioxidant enzymes and proteins such as glutathione-S-transferase (GST), super oxygen dehydrogenises (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione (GSH). When the balance between reactive oxygen species (ROS) production and antioxidant defense is lost oxidative stress results; which through a series of events deregulates the cellular functions leading various pathological conditions[7]. Recently, the hepatotoxic effects of gentamycin has been further explained by the discovery of increased levels of some liver marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and billirubin after administration of toxic dose of gentamycin in rats[8,9]. The present study aims to the ameliorative effect of dietary inclusion of garlic on gentamycin-induced hepatotoxicity in rats.

2. Materials and methods

2.1. Materials

Cured bulbs of garlic (*Allium sativum*) were purchased from Oja Oba market in Akure, Ondo State Nigeria. The authentication of the sample was done at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. These samples were sun dried, ground into fine powder and stored in an air-tight container. All chemicals and reagents used were of analytical grades and the water used was glass distilled. All the kits used for bioassays were sourced from RANDOX Laboratories Ltd., Crumlin, Co. Antrim, UK. Gentamycin was sourced as Enagent Gentamycin Sulphate Injection (Zhejang, Jinling Tiafeng Pharmaceutical Factory, Chezhan Road, Huzhou City, Zhejiang, China). Diet ingredients were purchased from VITAL Feeds, Jos, Nigeria.

2.2. Animals

Male albino rats weighing 97- 121 g used for this experiment were purchased from the breeding colony of a private farm in Akure, Ondo State Nigeria. The rats were maintained at 25 °C on a 12 h light/dark cycle with unrestricted access to food and water. They were acclimatized under these conditions for two weeks prior to the commencement of the experiments. This study received the approval of the institution's Ethical Committee on the use of laboratory animals and the animals were handled in accordance with NIH guide for the care and use of laboratory animals.

2.3. Experimental design and induction of hepatotoxicity

After acclimitation, the rats were randomly divided into four groups of six animals each. Group 1 and Group 2 were fed basal diet (50% skimmed milk, 36% corn starch, 10% groundnut oil and 4% mineral and vitamin premix) following a slightly modified method of Oboh *et al*.[10], Groups 3 and 4 were fed basal diet supplemented with 2% and 4% garlic respectively for 27 days prior to gentamycin (100 mg/kg body weight *i.p*..) administration which lasted for 3 d and the experiment was terminated after 30 d[11]. The rats were subjected to an overnight fast after which they were decapitated by cervical dislocation. The blood was rapidly collected into separate EDTA bottles by direct heart puncture and centrifuged at 3 000 r/min for 10 min to separate the plasma. Similarly, the livers were isolated, rinsed in cold saline (0.9% NaCl) and homogenized in phosphate buffer (pH 6.9). The homogenates were centrifuged at 7500 r/min for 10 min to obtain the clear supernatant. The clear supernatant obtained was used for various biochemical assays[12].

2.4. Analytical procedures

Plasma AST, ALT, albumin, triglyceride, total cholesterol, low density lipoprotein (LDL)-cholesterol and high density lipoprotein (HDL)-cholesterol were determined using commercially available kits (Randox Laboratories, UK). Lipid peroxidation was determined by thiobarbituric acid reaction[13]and quantified as malondialdehyde (MDA) content. SOD activity was determined by the method of Alia *et al*.[14], CAT activity was determined according to Sinha[15], reduced GSH content was assayed according to Ellman[16], and GST activity was determined according to the method of Habig *et al*[17]. Liver vitamin C content was determined according to Benderitter *et al*. and total protein was determined according to Lowry *et al*[18,19].

2.5. Data analysis

The results of replicate readings were pooled and expressed as mean±SD. One way analysis of variance was used to analyze the results and Duncan multiple test was used for the post hoc[20]. Statistical package for Social Science (SPSS) 16.0 for Windows was used for the analysis. The significance level was taken at $P<0.05$.

3. Results

Table 1 shows the average weight gain/loss in rats prior and after gentamycin (100 mg/kg) injection. There was a significant ($P<0.05$) weight loss in the gentamycin treated groups as compared to the Normal rats. Nevertheless, diets containing either 2% or 4% garlic inclusion were able to ameliorate the observed weight loss in gentamycin treated groups.

As observed in Table 2, control rats administered gentamycin and fed basal diet (Group 2) have significantly ($P<0.05$) elevated plasma activity levels of liver damage marker enzymes such as AST and ALT compared to normal rats fed basal diet (Group 1) but were found to return to normal levels upon treatment with diets containing either 2% or 4% garlic inclusions (Groups 3 and 4 respectively). However, control rats administered gentamycin and fed basal diet (Group 2) exhibited significantly ($P<0.05$) reduced plasma albumin level which was restored to near normal in diets containing either 2% or 4% garlic inclusion (Groups 3 and 4 respectively).

Furthermore, as revealed in Table 3, administration of gentamycin (100 mg/kg body weight) to the rats leads to reduction in the activities of plasma antioxidant enzymes (CAT, GST and SOD) relative to the normal rats (Group 1). However, feeding the gentamycin treated rats with diets containing either 2% or 4% garlic inclusions (Groups 3 and 4 respectively) caused a marked reversal in the depleted antioxidant enzymes level. Likewise, gentamycin treatment caused a marked decrease in plasma vitamin C content with a concomitant increase in MDA content of the treated rats. However, this trend was returned to near normal in the rats fed diets containing either 2% or 4% garlic inclusions (Table 3).

Table 4 depicts the effect of diets containing garlic inclusion on liver enzymatic antioxidant indices in gentamycin (100 mg/kg, *i.p.*) administered rats. Administration of gentamycin caused a significant ($P<0.05$) reduction in the activities of some liver antioxidant enzymes such as CAT, GST and SOD, with reversal of these trends to near normal in the rats fed diets containing either 2% or 4% garlic inclusions (Groups 3 and 4 respectively).

Furthermore, gentamycin administration also caused a

Table 1

Effect of diets supplemented with garlic on average weight gain/loss in gentamycin (100mg/kg *i.p.*) administered rats (g/rat).

Groups	Day 1	Day 27 (Prior to gentamycin injection)	Day 30 (3 d after gentamycin injection)	Weight gain (%)
1	97.3±36.6	170.6±29.9	192.9±39.9	13.1^{d}
2	120.8±52.2	167.3±31.1	148.9±29.6	-11.0^{a}
3	101.4±57.7	117.0±72.9	112.4±68.9	-3.9^{b}
4	140.9±97.7	171.4±73.4	168.5±73.1	-1.7^{c}

Values represent mean±SD ($n=6$). Values not sharing the same superscript letter are significantly ($P<0.05$) different.
Group 1: Normal rats fed basal diet, Group 2: Control rats administered gentamycin and fed basal diet, Group 3: gentamycin administered rats fed diet supplemented with 2% garlic, Group 4: gentamycin administered rats fed diet supplemented with 4% garlic.

Table 2

Effect of diets supplemented with garlic on liver function marker enzymes (ALT and AST) and albumin in gentamycin (100mg/kg, *i.p.*) administered rats.

Groups	Treatments	ALT (IU/L)	AST (IU/L)	Albumin (mg/dL)
1	Normal rats (basal diets)	15.9 ± 0.3^{a}	221.4 ± 6.7^{b}	3.7 ± 0.2^{ab}
2	Control rats (gentamycin treated rats+basal diets)	19.7 ± 0.5^{a}	230.0 ± 4.8^{c}	2.9 ± 0.3^{a}
3	Gentamycin treated rats+garlic (2%)	19.2 ± 0.7^{a}	236.5 ± 3.9^{d}	3.3 ± 0.7^{b}
4	Gentamycin treated rats+garlic (4%)	13.3 ± 0.1^{a}	208.5 ± 2.7^{a}	3.2 ± 0.4^{b}

Values represent mean±SD ($n=6$). Values not sharing the same superscript letter are significantly different ($P<0.05$).

Table 3

Effect of diets supplemented with garlic on plasma enzymatic antioxidants, Vitamin C content and MDA content in gentamycin (100 mg/kg, *i.p.*) administered rats.

Groups	Treatments	Enzymatic antioxidants (units/g protein)			Vitamin C content (mmol/mg protein)	MDA content (mmol/mg protein)
		CAT activity	GST activity	SOD activity		
1	Normal rats (basal diets)	4.1 ± 0.6^{b}	252.1 ± 3.7^{a}	7.7 ± 0.4^{b}	1.50 ± 0.03^{b}	1.30 ± 0.04^{a}
2	Control rats (gentamycin treated rats+basal diets)	2.9 ± 0.3^{a}	217.8 ± 1.0^{a}	2.9 ± 0.2^{a}	1.40 ± 0.04^{a}	1.60 ± 0.02^{a}
3	Gentamycin treated rats+garlic (2%)	3.2 ± 0.3^{ab}	243.4 ± 5.5^{a}	4.0 ± 0.3^{a}	1.30 ± 0.01^{a}	1.30 ± 0.02^{a}
4	Gentamycin treated rats+garlic (4%)	3.1 ± 0.3^{a}	236.0 ± 0.4^{a}	3.3 ± 0.4^{a}	1.40 ± 0.03^{ab}	1.10 ± 0.07^{a}

Values represent mean±SD ($n=6$). Values not sharing the same superscript letter are significantly different ($P<0.05$).

marked reduction in both liver vitamin C and GSH contents which was accompanied by an increase in the liver MDA content. However, these liver antioxidant indices were restored to near normal in rats fed diets containing either 2% or 4% garlic inclusions (Table 4).

The observed increase in the plasma atherogenic lipids (triglycerides and total cholesterol) and HDL-cholesterol, coupled with a concomitant decrease in the plasma HDL-cholesterol in gentamycin administered rats was reversed to near normal in diets containing either 2% or 4% garlic inclusions fed rats with the effect most prominent in 4% garlic included diets (Group 4) (Table 5).

Table 4

Effect of diets supplemented with garlic on liver enzymatic antioxidants, vitamin C content and MDA content and GSH content in gentamycin (100 mg/kg, *i.p.*) administered rats.

Groups	Treatments	Liver enzymatic antioxidants (units/100 g protein)			Liver non-enzymatic antioxidant (mmol/mg protein)		
		CAT activity	GST activity	SOD activity	Vitamin C content	MDA content	GSH content
1	Normal rats (basal diets)	30.1±0.5^{c}	198.9±5.1^{a}	25.6±0.8^{b}	0.89±0.02^{a}	3.19±0.20^{a}	0.210±0.002^{b}
2	Control rats (gentamycin treated rats+basal diets)	21.3±0.4^{b}	152.6±1.0^{a}	10.1±1.6^{a}	0.79±0.05^{a}	4.39±0.04^{b}	0.160±0.008^{a}
3	Gentamycin treated rats+garlic (2%)	19.9±0.2^{a}	181.2±2.5^{a}	12.6±0.3^{a}	0.81±0.04^{a}	3.24±0.10^{a}	0.130±0.001^{a}
4	Gentamycin treated rats+garlic (4%)	20.6±0.3^{b}	183.3±0.4^{a}	15.9±0.9^{a}	0.87±0.02^{a}	2.46±0.03^{a}	0.140±0.002^{a}

Values represent mean±SD (n=6). Values not sharing the same superscript letter are significantly different (P<0.05).

Table 5

Effect of diets supplemented with garlic on plasma lipid profile in gentamycin (100 mg/kg, *i.p.*) administered rats (mg/dL).

Groups	Treatments	Triglyceride	Total cholesterol	HDL-C
1	Normal rats (basal diets)	36.2±4.8^{d}	40.0±1.0^{a}	135.0±3.8^{b}
2	Control rats (gentamycin treated rats+basal diets)	54.0±2.7^{a}	56.0±1.4^{c}	145.8±6.8^{a}
3	Gentamycin treated rats+garlic (2%)	46.7±4.9^{c}	41.8±6.2^{a}	116.3±4.2^{c}
4	Gentamycin treated rats+garlic (4%)	50.0±1.7^{b}	45.1±3.6^{b}	99.7±1.9^{a}

Values represent mean±SD (n=6). Values not sharing the same superscript letter are significantly different (P<0.05).

4. Discussion

Results of this study confirmed that gentamycin at a dose of 100 mg/kg body weight produced significant hepatotoxicity as evidenced by which elevated activities of plasma ALT, AST and decrease level of albumin following gentamycin administration is in agreement with earlier studies[21]. AST presents two isoezymes, one located in the cytoplasm and the other in the mitochondria. The presence of these enzymes outside the cell represents damage to the hepatic cell. Albumin is a key component of serum proteins synthesized in the liver. The observed decrease in the plasma albumin level following gentamycin administration is attributed to the destruction of hepatic protein synthesizing sub-cellular structures. Nevertheless, the reversal of these plasma parameters as seen in the groups fed diets supplemented with garlic alludes to the possibility of the restoration of the normal functional status of the damaged liver, suggesting the hepatoprotective properties of garlic.

Certain drugs may induce oxidative stress by forming drug-derived radicals that can not only deplete the antioxidant defenses but can also react directly with biomolecules. Aminoglycosides can cause nephrotoxicity as well as hepatotoxicity. Aminoglycoside has been reported to alter activities of antioxidant enzymes such as SOD, CAT, GSH peroxidase, GST in various tissues[22]. The reduced enzyme activities in the gentamycin group are a generalized response, not specific to one enzyme which indicates several impaired function at several steps of the antioxidant pathway. It was postulated that aminoglycoside induced free radical generation and alteration in antioxidant enzymes activities may be the cause of tissue injury.

The experiment, however have clearly demonstrated the ability of gentamycin to induce oxidative stress in rat liver and plasma, as evidenced by the significant rise in MDA (lipid peroxidation product) coupled with significant decline in the endogenous antioxidants GST, SOD and CAT. This finding however, is consistent with earlier reports[23]. The increased lipid peroxidation lead to inactivation of the enzymes by crosses linking with MDA; this will cause an increased accumulation of superoxide, H_2O_2 and hydroxyl radicals which could further stimulate lipid peroxidation. This mechanism has a clue from work of Heeba and Basappa *et al*[24,25]. Decrease of antioxidant enzyme may be due to rapid consumption and exhaustion of storage of this enzyme in fighting free radicals generated during the development of hepatotoxicity. Reduced activity of one or more antioxidant systems, due to the direct toxic effect of gentamycin or volume depletion due to gentamycin administration, leads to an increase in lipid peroxidation. The decreased amount of intracellular glutathione and the accumulation of H_2O_2 and hydroxyl radicals are the triggering factors in gentamycin hepatotoxicity. Also, a highly significant decrease SOD and catalase activity was reported in this study, these observations are in agreement with those of, Yaman and Balikci[23]. Reduced glutathione act as an intracellular free radical scavengers and protect cells against radical mediated lipid peroxidation[26].

Furthermore, it was found that both the liver and plasma vitamin C content showed a highly significant decrease in

gentamycin treated rats as compared to normal rats. This result is in accordance with those of previous investigators[27]. The observed decrease in the levels of ascorbic acid may be due to their increased utilization for scavenging gentamycin and/or oxygen derived radicals. Vitamin C might ameliorate oxidative damage by decreasing lipid peroxidation and altering antioxidant defense system[28]. Ascorbic acid may affect the development of atherosclerosis and the onset of acute coronary events by several molecular mechanisms; it helps in maintaining arterial integrity, it can alter cholesterol metabolism by modulating the lipoprotein lipase activity[29].

Hypercholesterolemia and hypertriglyceridemia are risk factor for predicting coronary heart disease[30]. The present study demonstrates an increase in the level of triglycerides and cholesterol but there was no significant changes with HDL-C level of gentamycin-administered rats. HDL plays an essential role in the transport of cholesterol to the liver for excretion into bile. Furthermore, impaired hepatic function may also have affected cholesterol metabolism leading to hypercholesterolemia and hypertriglyceridemia. While the observed improvement in the lipid/cholesterol homeostasis in the garlic supplemented diet fed group might be due to the hypocholesterolemic and hypolipidemic properties of garlic[31-33]. In addition, improvement in the liver function on the other hand could have contributed to this observed lipid homeostasis.

Body weight is one important indicator of adverse effects of xenobiotics and it is considered a determinant parameter of toxicity testing. A very highly significant decrease in body weight gain observed in gentamycin intoxicated control rat. These results were in agreement with Bello and Chika study[34]. However, the observed increase in the body weight of the garlic supplemented diets fed group may be due to garlic induced increase in appetite. Rats treated with garlic (Groups 3 and 4) showed highest per cent increase in body weight when compared with other groups.

In conclusion, the study demonstrated that administration of gentamycin induces hepatic damage in rats. However, garlic supplemented diets ameliorate this gentamycin-induced hepatotoxicity through improvement in the rats' antioxidant status and modulating oxidative stress. Consequently, dietary inclusion of garlic may be a cheap management strategy in the management of acute hepatotoxicity or gentamycin-induced liver damage.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This study is supported by Federal University of Technology, Akure Staff Development Grant No.: 2012.

Comments

Background

The hepatotoxic nature of gentamycin is well documented. The use of antioxidant vitamins and natural products to ameliorate the hepatotoxic effects of gentamycin has also been demonstrated (Esmatparast and Amniattalab, 2008; Noorani *et al.*, 2010). The finding that gentamycin causes oxidative stress (Randjelovic *et al.*, 2012) limits its therapeutic usefulness. Garlic has been shown to possess profound antioxidants properties (Lawal *et al.*, 2011) and its use in this study to ameliorate the hepatotoxic and oxidative effects of gentamycin is instructive.

Research frontiers

Other researchers have examined the hepato-protective, nephron-protective and anti-oxidant effects of natural products on gentamycin in animal models. In fact a study has examined the nephron-protective and antioxidant effects of garlic in gentamycin treated rats (Pedraza-Chaverrí *et al.*, 2000). As far as I know, this is the first time the hepato-protective effect of garlic has been examined.

Related reports

Most of the related reports such as, Karatan *et al.*, 2005, Esmatparast and Amniatta lab, 2008 and Randjelovic *et al.*, 2012, support the finding of this study that natural products especially those containing antioxidants are protective against gentamycin induced hepatotoxicity, as well as nephrotoxicity.

Innovations and breakthroughs

Since the effect of garlic on the hepatotoxicity of gentamycin is relatively scarce, this study fills a gap in knowledge.

Applications

The use of garlic or any other readily available natural product to counter the hepato- and nephron-toxicity of gentamycin would substantially improve its therapeutic effects by reducing its side effects.

Peer review

This is a good study since it actually fills a gap in knowledge. The methodology is standard and presentation of results is adequate.

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