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Retention of testicular integrity and testosterone levels upon ingestion of garlic cloves (*Allium sativum*) in the Sprague-Dawley rat

Adejoke Elizabeth Memudu[1*△], Ibukun Dorcas Akinrinade[1△], Olalekan Michael Ogundele[2]

[1]*Department of Anatomy, College of Medicine, Bingham University, P.M.B 005, Karu, Nasarawa State, Nigeria*

[2]*Neural Systems Lab, Department of Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, 70802, LA, USA*

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ABSTRACT

Objective: To investigate the effects of acute and chronic aqueous garlic extract ingestion on testicular cellular integrity and serum testosterone levels.

Methods: Twenty (20) male Sprague-Dawley rats weighing an average of 120 g were used. Animals were divided into three groups. Group A served as control (10 rats for 28 and 56 d respectively), while treatment Groups B and C were given 200 mg/kg for *Allium sativum* (garlic cloves) extract for 28 and 56 d respectively.

Results: Histological analysis revealed the presence of all spermatogenic lineages, appearance of proliferative activities in the interstitial cells, as well as increased serum testosterone levels.

Conclusions: This study confirmed proliferative and restorative potentials in both acute and chronic garlic ingestion.

1. Introduction

The use of medicinal plants for medicinal remedies has been documented since ancient times and they provide a useful source of new therapeutics[1,2].

Garlic belongs to the family Liliaceae. Its leaves collections (bulb), which can be divided into cloves, are used for culinary and medicinal purposes. It has a characteristic pungent and spicy flavor[3]. Garlic is made up of the important bioactive components such as allicin (the active compound responsible for its hot sensation), ajoene, enzymes, water, vitamin B, minerals and flavonoids[4].

Garlic has been reported to be capable of human blood pressure regulation[5,6], glucose level regulation, platelet aggregation[7], hyperlipidemia, and the reduction of atherosclerosis building up in the arterial system[8,9]. Also, it has been reported to act as an anti-viral, anti-bacterial and anti-candida agent, and it has been implicated in cancer prevention due to the presence of diallyl disulfide in its composition[10,11]. Furthermore, it is said to reduce hypercholesterolemia[12] and to boost testosterone levels[13].

However, the reported side effects of excessive garlic consumption have been found to include halitosis (non-bacterial), nausea, emesis, diarrhoea/gastrointestinal discomfort, bleeding, sweating, *etc*. Also, the prolonged feeding of high doses of raw garlic to experimental rats will result in anaemia, weight loss and stunted growth due to red blood cells lysis[14].

United States Department of Agriculture nutrient database states that 100 g of raw garlic contains carbohydrates, dietary fibre, fat, protein, β-carotene, thiamin (Vit B1), riboflavin (Vit B2), niacin (Vit B3), pantothenic acid (Vit B5), adermin (Vit B6), folate (Vit B9), vitamin C, calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, manganese and selenium[4].

While some reports show that heated garlic juice is effective in the recovery of testicular functions after experimental testicular hypogonadism[3,15,16], other studies have reported that powdered garlic preparations impair testicular and male reproductive functions[17,18]. Garlic metabolites such as diallyl trisulfide have been reported to have spermicidal effects[19]. Chronic administration of garlic powder (50 mg/d) was said to result in the inhibition of spermatogenesis in rats[17], and a study showed that 10%, 15% and 20% of the crude extracts respectively decreased serum testosterone levels[20].

The contrasting findings in the mechanisms of garlic actions on testosterone activities and testicular integrity create the need for further research[1,18,21,22]. Hence, this study was designed to evaluate the acute and chronic effects of 200 mg/kg of aqueous garlic extract

*Corresponding author: Adejoke Elizabeth Memudu, Department of Anatomy, College of Health Sciences, Bingham University, P.M.B. 005, New Karu, 961106, Nasarawa State, Nigeria.
Tel: +2348051694169
E-mail: jokememudu@gmail.com
△These authors contributed equally to this work.

(AGE) on the testicular weight, testosterone level and testicular cytoarchitecture.

2. Materials and methods

2.1. Experimental animals

Twenty (20) male Sprague-Dawley rats with average age of 2 months and average weight of 120 g were used for this study. Animals were kept in well ventilated cages in the animal holding of the Department of Anatomy, University of Lagos, Nigeria, under standard laboratory conditions [12 h light/dark cycle (lights on: 8:00 am), temperature at 29 °C, humidity at 50%] and given pelleted rat feed (UAC, Vital Feeds Lagos, Nigeria) and water *ad libitum*. They were cared for in accordance to the ethical regulations in the Guide for the Care and Use of Laboratory Animals[23]. The animals were allowed to acclimatize for 2 weeks before experimentation.

2.2. Plant material

Fresh garlic [*Allium sativum* (*A. sativum*)] bulbs were procured from Lawanson market, Surulere, Lagos, Nigeria. They were authenticated in Botany Department in University of Lagos, Lagos, Nigeria, where voucher specimen was deposited (Figure 1A).

2.2.1. Preparation of the aqueous extracts of each spice

A. sativum (garlic cloves) were separately sun-dried and crushed into powdered by using a blender (Figure 1B). The dried powder was weighed (10 g) and macerated in 50 mL of distilled water. Prepared extract was stored in a refrigerator and each animal was given the aqueous extract of *A. sativum* via gastric intubation using an oral cannula.

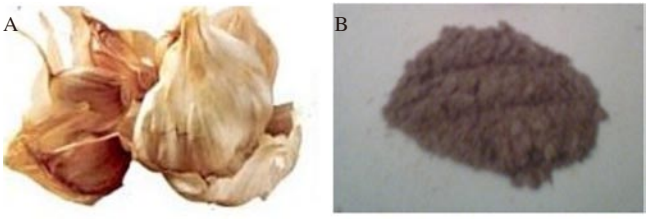

Figure 1. Representative image of *A. sativum* in raw form (A) and in its dried grinded (B) used in this study[24].

2.3. Experimental protocol

Rats were divided into four groups. Group A (control group) received feed pellets and water *ad libtum*. Group B received 200 mg/kg of the extract for 4 weeks (28 d). Group C received 200 mg/kg of the extract for 8 weeks (56 d). Administration was done once daily between 08:00–09:00 am.

2.4. Euthanasia of experimental animals

Experimental animals were euthanized 24 h after the last administration and final weights were recorded. Euthanization was done by giving an intraperitoneal injection of 50 mg/L of ketamine (Claris Lifesciences Ltd., India).

2.5. Histology

Incision was made in the lower abdominal wall and the testes were excised. Organ weights were taken using an analytical weighing scale (Haerus, Germany) after which organs were stored in Bouin's fluid for fixation. One half of the longitudinal sectioned testes was processed for histological studies, embedded in paraffin wax and serially sectioned using a Leica rotary microtome (Leica biosystems, Germany) set at 5 μ. Testicular tissue sections were stained using haematoxylin and eosin stain for general cytoarchitectural examination.

2.6. Statistical analysis

Data were analyzed using SPSS statistical software and Microsoft Excel. The statistical analysis was carried using repeated measures for weight changes and One-way analysis of variance for testicular weight and serum testosterone levels. Fisher's least significant difference was used for multiple mean comparison using statistica software. Data were expressed as mean±SEM. $P<0.05$ was considered significant.

3. Results

3.1. Body weight changes

An overall increase in final body weight was seen in both control and experimental groups at the end of the experiment ($P<0.05$) in both 28 and 56 d when compared with their respective initial body weights (Figure 2). However, at the end of the experiment, there was no statistically significant ($P>0.05$) final body weight change in Group B when compared with the control group. In contrast, there was a significant reduction in final body weight ($P<0.000\,1$) in Group C when compared with the control group.

3.2. Testicular weight changes

An overall increase was shown in the testicular weights in all experimental groups when compared with the control group (Figure 3). Group B animals showed statistically significant testicular weight increase when compared with the control group ($P<0.05$), likewise Group C animals showed statistically significant testicular weight increase ($P<0.05$) when compared with the control group animals. Noteworthy, Group C animals had statistically significant testicular weight increase ($P<0.01$) when compared with Group B animals (Figure 3).

3.3. Serum testosterone levels

Serum testosterone level was increased in the experimental Groups B and C in comparison with the control group (Figure 4). There was a statistically significant overall increase in testosterone levels in all experimental groups compared with control group ($P<0.05$). Indeed, *post hoc* analysis showed a statistically significant increase in testosterone levels ($P<0.05$) in Group B when compared with the

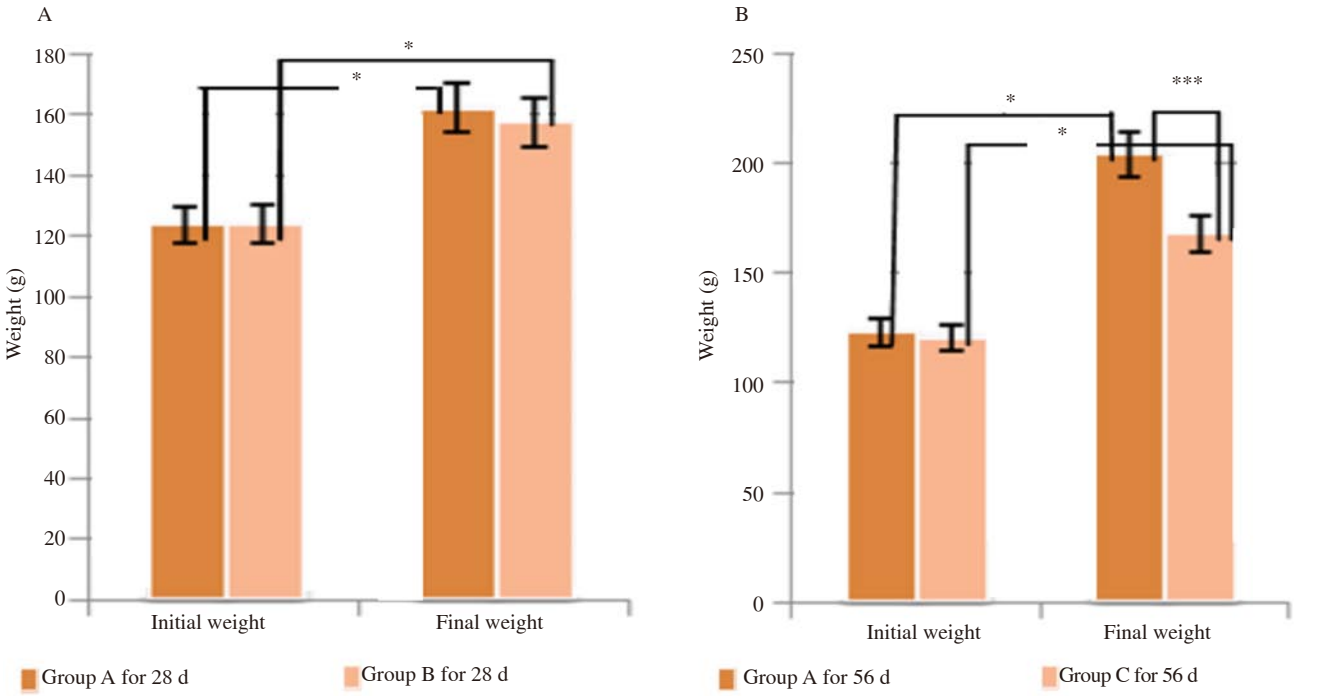

Figure 2. Mean values of initial and final body weight measurements of the experimental animals following 28 (A) and 56 (B) d of AGE treatments respectively.

*P<0.05 represents statistically significant increase in final body weights in both control (Group A) and experimental groups (B,C) in comparison with the initial body. ***P<0.001 represents statistically significant decrease in final body weight of Group C animals in comparison with the control group.

control group, likewise, there was a statistically significant increase in Group C when compared with the control group animals (P<0.05). Moreover, there was a significant increase in serum levels (P<0.01) in the Group C animals when compared with Group B animals.

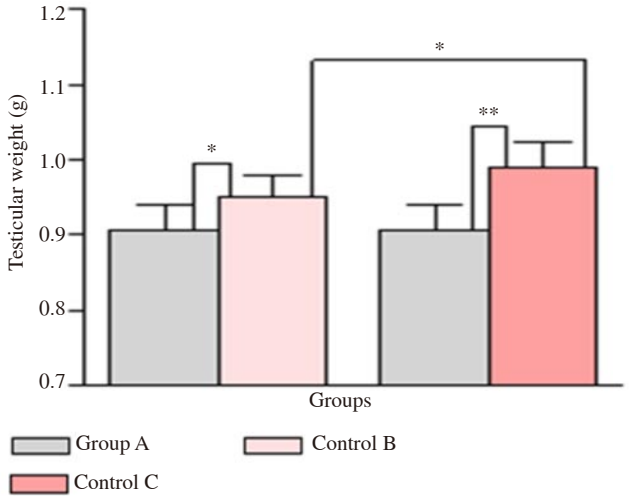

Figure 3. Mean values for testicular weights of experimental animals treated with AGE for 28 and 56 d respectively.

*P<0.05 represents significant increase in testicular weight after 28 d treatment with AGE in comparison with control group. **P<0.01 represents significant increase in testicular weight after 56 d treatment with AGE in comparison with control group. A statistically significant testicular weight increase was shown in Group C compared with Group B animals (*P<0.05).

3.4. Histological results

In correlation with serum testosterone levels, there was appearance of morphological changes in the experimental groups, an indication of proliferative activities taking place in these treatment groups (Figures 5B and 5D) when compared with the control groups (Figures 5A and 5C). The testicular cytoarchitecture of all the groups showed relatively distinct seminiferous tubules bounded together by loose interlobular connective tissue and groups of interstitial cells or leydig cells. In the experimental Group B (Figure 5B), though lumen appeared slightly wider, all cells spermatogenic lineage appeared present, also leydig cells appeared intact. Representative image of Group C animals treated for 56 d showed intact testicular histoarchitecture, with the appearance of all cells of spermatogenic lineage in the germinal epithelium. Notably, the appearance of spermatids indicated spermatogenic processes at the advanced stages of the spermatogenic cycles (Figure 5D).

4. Discussion

This study demonstrates the supportive role of garlic in body weight regulation and maintenance of testicular integrity and function.

Garlic supplementation has been implicated in body weight regulation[10,25]. This study supports the role of garlic in body weight regulation during both acute and chronic garlic intake, thus, even though the final body weight of both control and experimental animals increased when compared with their initial body weights, there was a dose dependent response in their final body weights, and this was evident by the lack of significant changes in final body weights during acute garlic ingestion when compared with the control animals and the profound decrease in final body weight during chronic garlic ingestion. The regulatory role of garlic in body weight regulation is supported by previous findings[26-28]. Furthermore, this study emphasizes the dose dependent response of garlic intake on weight regulation.

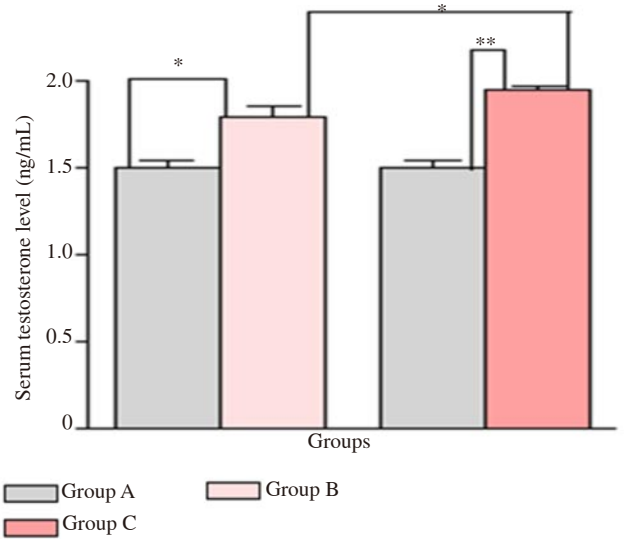

Figure 4. Mean serum testosterone levels for the AGE treated rats in comparison control group for 28 and 56 d respectively.
$^{*}P<0.05$ represents significant increase in serum testosterone after 28 d treatment with AGE in comparison with control group. $^{**}P<0.01$ represents significant increase in serum testosterone after 56 d treatment with AGE in comparison with control group. A statistically significant serum testosterone increase was shown in Group C compared with Group B animals ($^{*}P<0.05$).

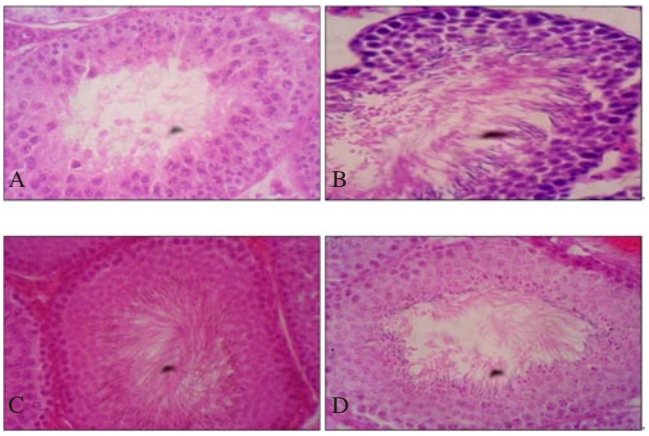

Figure 5. Representative images of haematoxylin and eosin stained testicular sections (×400).
Control sections A, B showed appearance of all spermatogenic cells in the seminiferous tubule, with intact leydig cells and clearly distinct lumen and basement membrane; C, D images represented Groups B and C given AGE for 28 and 56 d respectively. Cells have similar cytoarchitectural pattern as the control group.

Garlic oil and the diallyl disulfide components in garlic have been implicated in weight gain regulation[25]. It has been proposed that garlic regulates body weight due to its ability to decrease lipid in adipose tissues via activation of increased levels of uncoupling proteins 1 and 2 required for fat oxidation thereby causing a decreased weight gain[29]. Another report claims that garlic possesses antiobesity properties due to its ability to mediate activation of AMP kinase which causes increased thermogenesis and decreased expression of the multiple genes involved in adipogenesis by a decrease in mRNA level in adipogenic genes in white adipose tissue[10].

This study also confirms an increase testicular weight of the animals exposed to acute and chronic garlic ingestion. Notably, a more profound increase in testicular weight was found during chronic garlic administration. The major components of garlic are zinc and selenium. These substances have been reported to play regulatory roles in the testicular activities especially steroidogenic enzymes production[4,30].

The testes are involved in spermatogenesis, a process in which spermatogenic cells are formed; testosterone (androgen) deficiency negatively affects spermatogenesis[31] by altering spermatid-Sertoli cell junctions, thus resulting in the premature detachment of round spermatids from Sertoli cells and spermatogenic epithelium[28,32]. In this study, garlic was found to play a proliferative role in testosterone secretion such that an increase in testosterone secretion is shown in both acute and chronic garlic intake. Moreover, a dose dependent activity was found such that chronic garlic intake corresponded with higher serum testosterone levels. This is contrary to some findings that report crude garlic extracts causing reduction in serum testosterone levels. The disparity in results obtained may be due to the use of varied dose of administration as well as varied duration of garlic exposure[17,20,33,34].

Testosterone plays an important role in the spermatogenic cell proliferation. The regulatory role of garlic in testosterone secretion was further confirmed in the histological analysis for both the control and experimental groups such that there was the appearance of intact spermatogenic cells, Leydig cells, well aligned lumen and clearly defined cellular membranes. This contradicts some findings that report degenerative changes in the testes following the administration of 1.4 and 2.4 mL of 100 mg/L garlic extract to rats[18,34], but supports some other findings that report garlic as having protective and proliferative functions in the testes[35-37]. Further studies are required to confirm the molecular mechanisms mediating these supportive and regulatory roles of garlic in testicular function.

The data from this present study confirm the regulatory role of garlic in regulating body weight, organ weight, serum testosterone levels and testicular integrity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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References

[1] Abdelmalik SW. Histological and ultrastructural changes in the adult male albino rat testes following chronic crude garlic consumption. *Ann Anat* 2011; **193**(2): 134-141.

[2] Siew YY, Zareisedehizadeh S, Seetoh WG, Neo SY, Tan CH, Koh HL. Ethnobotanical survey of usage of fresh medicinal plants in Singapore. *J Ethnopharmacol* 2014; **155**(3): 1450-1466.

[3] Kasuga S, Uda N, Kyo E, Ushijima M, Morihara N, Itakura Y. Pharmacologic activities of aged garlic extract in comparison with other garlic preparations. *J Nutr* 2001; **131**(3s): 1080S-1084S.

[4] Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, et al. The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. *Curr Biol* 2005; **15**(10): 929-934.

[5] Silagy CA, Neil HA. A meta-analysis of the effect of garlic on blood pressure. *J Hypertens* 1994; **12**(4): 463-468.

[6] Ried K, Frank OR, Stocks NP, Fakler P, Sullivan T. Effect of garlic on blood pressure: a systematic review and meta-analysis. *BMC Cardiovasc Disord* 2008; **8**: 13.

[7] Chan KC, Yin MC, Chao WJ. Effect of diallyl trisulfide-rich garlic oil on blood coagulation and plasma activity of anticoagulation factors in rats. *Food Chem Toxicol* 2007; **45**(3): 502-507.

[8] Koscielny J, Klüssendorf D, Latza R, Schmitt R, Radtke H, Siegel G, et al. The antiatherosclerotic effect of *Allium sativum*. *Atherosclerosis* 1999; **144**(1): 237-249.

[9] Yeh YY, Lim HS, Yeh S, Picciano MF. Garlic extract attenuates hyperhomocysteinemia caused by folic acid deficiency in the rat. *Nutr Res* 2005; **25**(1): 93-102.

[10] Lee MS, Kim IH, Kim CT, Kim Y. Reduction of body weight by dietary garlic is associated with an increase in uncoupling protein mRNA expression and activation of AMP-activated protein kinase in diet-induced obese mice. *J Nutr* 2011; **141**(11): 1947–1953.

[11] Galeone C, Pelucchi C, Levi F, Negri E, Franceschi S, Talamini R, et al. Onion and garlic use and human cancer. *Am J Clin Nutr* 2006; **84**(5): 1027-1032.

[12] Mahmoodi M, Islami MR, Asadi Karam GR, Khaksari M, Sahebghadam Lotfi A, Hajizadeh MR, et al. Study of the effects of raw garlic consumption on the level of lipids and other blood biochemical factors in hyperlipidemic individuals. *Pak J Pharm Sci* 2006; **19**(4): 295-298.

[13] Oi Y, Imafuku M, Shishido C, Kominato Y, Nishimura S, Iwai K. Garlic supplementation increases testicular testosterone and decreases plasma corticosterone in rats fed a high protein diet. *J Nutr* 2001; **131**(8): 2150-2156.

[14] Augusti KT. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). *Indian J Exp Biol* 1996; **34**(7): 634-640.

[15] Olalekan Lawal A, Lawal AF, Ologundudu A, Adeniran OY, Omonkhua A, Obi F. Antioxidant effects of heated garlic juice on cadmium-induced liver damage in rats as compared to ascorbic acid. *J Toxicol Sci* 2011; **36**(5): 549-557.

[16] Park JH, Park YK, Park E. Antioxidative and antigenotoxic effects of garlic (*Allium sativum* L.) prepared by different processing methods. *Plant Foods Hum Nutr* 2009; **64**(4): 244-249.

[17] Dixit VP, Joshi S. Effects of chronic administration of garlic (*Allium sativum* Linn.) on testicular function. *Indian J Exp Biol* 1982; **20**(7): 534-536.

[18] Hammami I, Nahdi A, Atig F, Kouidhi W, Amri M, Mokni M, et al. Effects of garlic fractions consumption on male reproductive functions. *J Med Food* 2013; **16**(1): 82-87.

[19] Chakrabarti K, Pal S, Bhattacharyya AK. Sperm immobilization activity of *Allium sativum* L. and other plant extracts. *Asian J Androl* 2003; **5**(2): 131-135.

[20] Hammami I, Amara S, Benahmed M, El May MV, Mauduit C. Chronic crude garlic-feeding modified adult male rat testicular markers: mechanisms of action. *Reprod Biol Endocrinol* 2009; **7**(1): 65.

[21] Hfaiedh N, Murat JC, Elfeki A. Protective effects of garlic (*Allium sativum*) extract upon lindane-induced oxidative stress and related damages in testes and brain of male rats. *Pestic Biochem Phys* 2011; **100**(2): 187-192.

[22] Hammami I, El May MV. Impact of garlic feeding (*Allium sativum*) on male fertility. *Andrologia* 2013; **45**(4): 217-224.

[23] National Institutes of Health. *Guide for the care and use of laboratory animals*. DHEW publication (NIH), revised. Bethesda: Office of Science and Health Reports, DRR/NIH; 1985.

[24] Grasindo P. *Essential atlas botany*. New Delhi: Sterling Publishers; 2007.

[25] Sheen LY, Chen HW, Kung YL, Liu CT, Lii CK. Effects of garlic oil and its organosulfur compounds on the activities of hepatic drug-metabolizing and antioxidant enzymes in rats fed high- and low-fat diets. *Nutr Cancer* 1999; **35**(2): 160-166.

[26] Chen HW, Yang JJ, Tsai CW, Wu JJ, Sheen LY, Ou CC, et al. Dietary fat and garlic oil independently regulate hepatic cytochrome p(450) 2B1 and the placental form of glutathione S-transferase expression in rats. *J Nutr* 2001; **131**(5): 1438-1443.

[27] Kim I, Kim HR, Kim JH, Om AS. Beneficial effects of *Allium sativum* L. stem extract on lipid metabolism and antioxidant status in obese mice fed a high-fat diet. *J Sci Food Agric* 2013; doi: 10.1002/jsfa.6094.

[28] Chen YC, Kao TH, Tseng CY, Chang WT, Hsu CL. Methanolic extract of black garlic ameliorates diet-induced obesity via regulating adipogenesis, adipokine biosynthesis, and lipolysis. *J Funct Foods* 2014; **9**: 98-108.

[29] Kim Y, Lee MS, Kim JS, Lee HS. Garlic decreases body weight via decrease of serum lipid and increase of uncoupling proteins mRNA expression. *FASEB J* 2007; **21**: lb277.

[30] Sadik NA. Effects of diallyl sulfide and zinc on testicular steroidogenesis in cadmium-treated male rats. *J Biochem Mol Toxicol* 2008; **22**(5): 345-353.

[31] Saito K, O'Donnell L, McLachlan RI, Robertson DM. Spermiation failure is a major contributor to early spermatogenic suppression caused by hormone withdrawal in adult rats. *Endocrinology* 2000; **141**(8): 2779-8275.

[32] Tesarik J, Martinez F, Rienzi L, Iacobelli M, Ubaldi F, Mendoza C, et al. *In-vitro* effects of FSH and testosterone withdrawal on caspase activation and DNA fragmentation in different cell types of human seminiferous epithelium. *Hum Reprod* 2002; **17**(7): 1811-1819.

[33] Hammami I, Nahdi A, Mauduit C, Benahmed M, Amri M, Ben Amar A, et al. The inhibitory effects on adult male reproductive functions of crude garlic (*Allium sativum*) feeding. *Asian J Androl* 2008; **10**(4): 593-601.

[34] Ebomoyi MI, Ahumibe KC. Serum testosterone and morphology of the testes in wistar rats following chronic garlic feeding. *J Physiol Pathophysiol* 2010; **1**: 39-43.

[35] Ola-Mudathir KF, Suru SM, Fafunso MA, Obioha UE, Faremi TY. Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. *Food Chem Toxicol* 2008; **46**(12): 3604-3611.

[36] Ponnusamy M, Pari L. Protective role of diallyl tetrasulfide on cadmium-induced testicular damage in adult rats: a biochemical and histological study. *Toxicol Ind Health* 2011; **27**(5): 407-416.

[37] Khalil WK, Ahmed KA, Park MH, Kim YT, Park HH, Abdel-Wahhab MA. The inhibitory effects of garlic and *Panax ginseng* extract standardized with ginsenoside Rg3 on the genotoxicity, biochemical, and histological changes induced by ethylenediaminetetraacetic acid in male rats. *Arch Toxicol* 2008; **82**(3): 183-195.