Journal of College of Education for pure sciences(JCEPS)
Web Site: http://eps.utq.edu.iq/ Email: com@eps.utq.edu.iq
Volume 7, Number 1, January 2017

Modulation Male Fertility in Diabetic Rats by Allicin Administration

Dr. Hazar Shakir Saleh[1];Dr. Enaas Saleh Al- Kayat[2]

hazarsaleh624@yahoo.com

[1] Unit ofCancer Research, College of Medicine, Thi-Qar University, Iraq

[2] Department of Obstetrics and Gynecology, College of Medicine, Thi- Qar University, Iraq

Abstract

Diabetes has been known to cause fertility complications including male reproductive organs failure and infertility. However, many studies have examined the anti-diabetic effect of major component of garlic compounds, allicin, in both types of diabetes mellitus (DM), but to date, the role of allicin in male fertility protection throughout disease process of diabetes mellitus remains unclear. Objective: Hence this study has been undertaken to evaluate histological changes via allicin administration in the maintenance male reproductive system function in rats-exposed to diabetes mellitus. Methods: Thirty adult male rats have been divided into three groups (10 rats in each group). The two control groups, including diabetic rats (DM induced by intraperitoneal injection with a single dose of Streptozocin (STZ) 65mg/kg body weight) and normal rats, which were injected with normal saline, once daily for 21 days. The third group received allicin. Blood glucose levels were tested every day through 21 days of the experimental period. At the end of the experiment, testis tissues were taken from the rats for histological examination and they were fixed in 10% neutral formalin solution and hematoxylin and eosin (H&E) stained sections of testis were prepared for histopathological and Histomorphometric examination under light microscope. Results: The results were compared to the ones obtained from healthy and non-treated diabetic rats as well as allicin -treated diabetic rats. Histopathological examinations of stained testicular tissue sections showed necrosis and vacuolization of spermatocytes to be reasonably reduced in the diabetic treated rats as well as demonstrated abnormal spermatogenises but it was found to restore the testicular histology and components of spermatogenises to near normal in the allicin treated group. Subsequent histomorphometrical evaluation also showed a reduction in the diameter and height epithelia of seminiferous tubules in the diabetic control rats which were significantly improved in the allicin- treated diabetic rats ($P < 0.05$) which were comparable to normal rats. Conclusion these results indicate that the active compound of garlic plant may help in the prevention of the complications of diabetes and confer essential protection for male reproductive function as serious complication.

Keywords: diabetic, fertility, rats, histopathological changes, garlic.

Introduction

Diabetes is an endocrine disorder resulting in metabolic disturbance that is characterized by hyperglycemia (Chandra et al., 2004). It has been reported that diabetes affects nearly 10% of the world population according a worldwide survey (Kar et al., 1999). In same line, International Diabetes Federation had demonstrated that 194 million people had incidence to diabetes in 2003, which will increase to 333 million by 2025 (Sicree et al., 2003). It is prospective; that is regarded as serious problem that threats to public health in the coming years.

Gradually, deleterious effects of processing diabetes lead to disturbance in function of various systems in the body. Accordingly, uncontrolled blood glucose is thought to be the main factor in the progress of diabetic complications in both type 1 and type 2 diabetes (American Association of Diabetes, 2002). Subsequently, it is well established that diabetes can produce evident complications in male reproductive system according to several studies that have determined partial infertility in male reproductive system. Most complications were documented such as erectile dysfunction and retrograde ejaculation in men (Rodriguez-Rigau, 1980).

Previous study has been conducted by Andersson et al (1994) which observed microscopic abnormalities in testicular morphology in 10 Type 1 diabetic young men with erectile dysfunction. In addition, determination of new analytical techniques recently provided as evidence in emerging of undetectable effects of diabetes on male fertility, showing serum testosterone impairment linked to increased or decreased serum levels of gonadotropins (Escrig et al, 2002; Ballester et al, 2004). Also In other reports, it has been found histopathological effects were present in diabetic compared with nondiabetic animals such as severe macroscopic testicular and epididymal structural lesions.

Furthermore, the direct effect of diabetes on testicular function being throughout significantly decreased seminiferous tubule diameter, increased testicular blood vessel numbers, and altered tubule stage distribution were determined in diabetes compared with controls (Cai et al., 2000; Amaral et al, 2006 and Navarro et al, 2010). That was revealed an increase in advanced glycated end products in diabetic men suggests that these compounds could play a hitherto unrecognized role in male infertility, as proposed by Mallidis et al (2009).

With regard to recently molecular examination, precisely, a recent work has established a significant increase in level of sperm nuclear DNA fragmentation in men with diabetes (Agbaje et al., 2008). A number of investigations, of oral anti- diabetic agents from plants used in traditional medicine, have been conducted and many of the plants were found with good activity (Kesari et al., 2007). The World Health Organization (WHO) has also recommended the evaluation of the plants' effectiveness in conditions where we lack safe, modern drugs (Day, 1998).

Several animal and human studies have shown that allicin may be a very valuable therapeutic agent for diabetes. Many studies have examined the hypoglycemic effect of allicin in both types of DM, (Ashraf et al., 2011), but till now the mechanism has not been discussed regarding DM in both types while the probable mechanism underlying Allium sativum and allicin hypoglycemic effects in type 2 diabetes most likely is increased insulin secretion and sensitivity (Mustafa et al., 2007;. Eidi et al., 2006). Researchers have postulated that Allicin lower glucose levels by competing with insulin (which is also a disulfide) for insulin-inactivating sites in the liver, resulting in an increase of free insulin (Lucy et al., 2002).

In addition to the activity garlic of components studies, a number of researchers have recently studied allicin; exert their anti-diabetic action by stimulating the insulin production and secretion by pancreas, interfering with dietary glucose absorption, and favoring the insulin saving (Thomson et al., 2007). Moreover, it has been suggested that allicin treatment with various doses that was given orally daily for long time, both in vivo and in vitro results demonstrated a favorable effect in reducing blood cholesterol blood glucose and triglycerides levels and caused a significant decrease in lowering the hepatic cholesterol storage (Lu et al., 2012). Accordingly, very limited studies are available in the literature regarding the preservation of male reproductive function by administration active ingredient of garlic, allicin, to relief this important complications of diabetic status.

Methodology

Animal Husbandry

Thirty adult male rats aged between 12 to 16 weeks of an average weight 150-250g were used in this study. They were housed in plastic cages in air conditioned house with temperature maintained at 18°C to 20°C and 12:12 hours light - dark cycle. Rats were fed with normal pellet and tap water was given to the mice added libitum. Rats have been divided into three groups (10 rats in each group) and given different treatment for 21consecutive days. The groups are classified according to the treatment they received as follows:

i)Group A- Normal control group used as normal untreated rats given 0.1 ml of normal saline by intraperitoneal injection daily for a period of 21days.

ii) Group B- rats were treated with a single dose of Streptozotocin (65mg/kg) body weight.

iii) Group C–DM rats received intraperitoneal injection dose of allicin of 16mg/kg BW for 21 days (Reinhart et al., 2008).

Diabetes was confirmed in STZ-treated rats by measuring fasting blood glucose levels three days after STZ was induced. Rats with marked hyperglycaemia (blood glucose level above 13.9mmol/L) were selected and used in the study (Mariee et al., 2009and Metwally et al., 2012). After the administration of the last dose the animals were fasted overnight and the following day all the animals were euthanized under anesthesia in a chamber containing diethyl ether. Both testis were eviscerated and grossed to prepare for histological processing steps.

Testes were cut in cross section, entirely submitted in one labeled histology cassette. Each specimen was cut between 3-4 mm in thickness. It was immediately fixed in10% neutral formalin solution for 24 h, processed by using a graded ethanol series, and then embedded in paraffin. The paraffin –embedded blocks were sectioning to prepare slides and stained with H&E. All sections were examined for histopathological and Histomorphometric changes under light microscope. Furthermore, epithelial height and diameter of seminiferous tubules were determination by using image J software.

Statistical Analysis

Statistical analyses were using SPSS 19 software. All data were represented as mean±SD. The one-way analysis of variance (ANOVA) that was followed by Tukey's post-hoc test which were used for analysis in statistical significant differences at $p<0.05$and $p<0.001$ were considered.

Results

Histopathological observations

In the normal group, results showed that appearance of seminiferous tubules was completely healthy spermatogenic cells. Furthermore, it has been observed obvious developmental stages of spermatogenesis in lumen which were organized and the basement membrane thickness was also normal. Additionally, interstitial tissue space appeared intact (Figure 1).

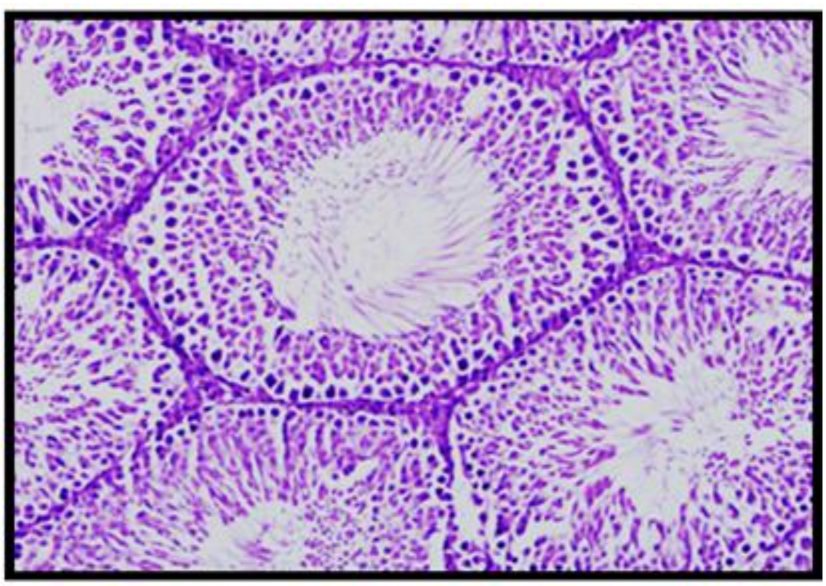

Figure 1. This microphotograph showing normal structural architecture of testicular tissue and seminiferous tubules filled with healthy spermatids. (X 10, E&H)

In the diabetic control group, the seminiferous tubules showing the spermatocytes and spermatids have been reduced. Cellular levels were undergoing from nuclear changes, karyolysis and severe necrotic changes due to disappear of nucleus; moreover the connections between cells were quite disappeared. It was Also observed increase the thickness of basement membrane meanwhile the spaces between seminiferous tubules were somewhat empty from interstitial tissue clear. Seminiferous tubules lumen, especially in the center filling with residue of dead cells was observed (Figure 2).

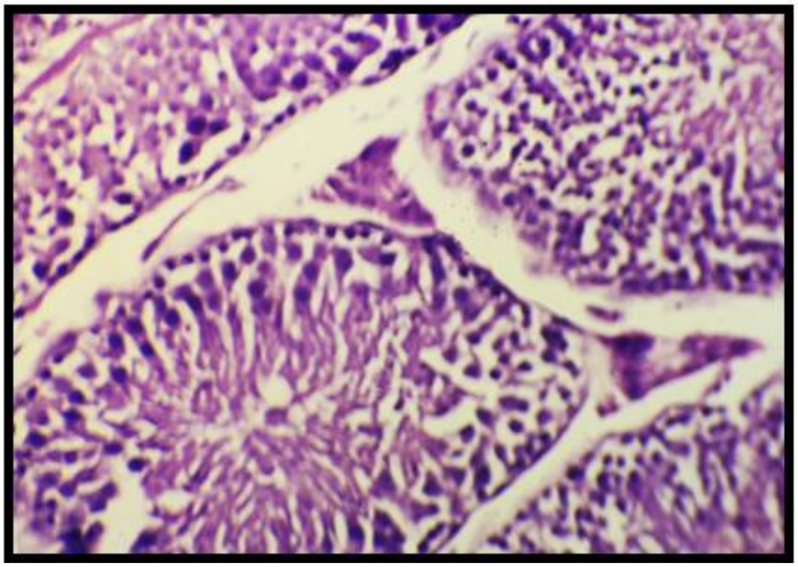

Figure (2). This microphotograph showing disorganization in architectural structure of testicular tissue and seminiferous tubules filled with dead spermatocytes and spermatids.. (X 10, E&H)

In term of allicin-treated diabetic group, there were no structural testicular defects. Seminiferous tubules presented all stages of spermatogenesis within tubules, which were in distinct developmental status. It was detected that the appearance of cellular levels were normally noticeable involving spermatocytes and spermatids. Also the thickness of the basement membrane was seen as in normal state that decrease in the thickness, Furthermore, the interstitial space between tubules was restored (Figure 3).

In this study, it was revealed that the first evaluation of spermatogenesis impairment throughout seminiferous tubule diameters which were significantly reduced in diabetic rats compared to control ($p<0.05$). Moreover, allicin-treated diabetic rats exhibited remarkable increase in seminiferous tubule diameters compared to diabetic rats ($p<0.05$) (Table 1).

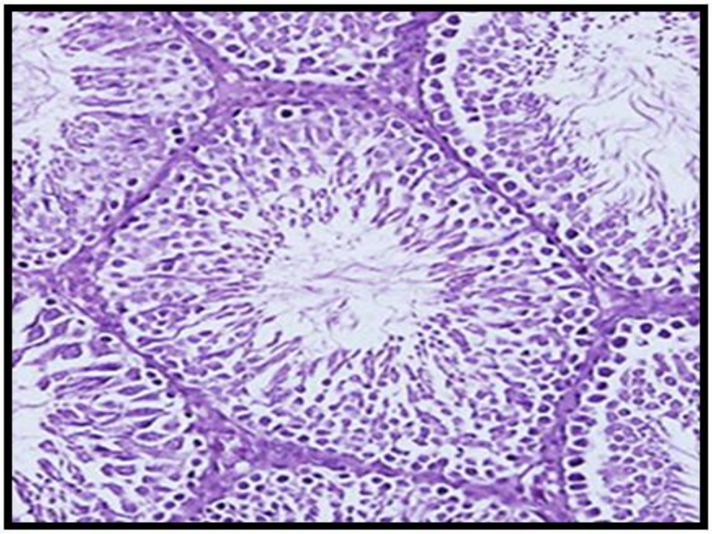

Figure (3). This microphotograph showing restoration in structural architecture of testicular tissue and seminiferous tubulesfilled with intact spermatocytes and spermatids. (X 10, E&H).

Table1. Showing the evaluation of allicin effect on spermatogenesis impairment in diabetic rats.

Groups	Epithelial Height (mm)	Diameter of Seminiferous Tubules (mm)
Normal group	58.42±0.9	14.58±0.31
Diabetic group	34.64±0.7	11.65±0.59
Allicin-treated group	55.78±0.7	13.69±0.45

Data were represented as mean ±SD. Number of animals in each group was ten.

As well as, it was observed a significant elevation ($p<0.001$) in epithelial height in the allicin-treated diabetic and control groups compared with the diabetic group (Figure 4).

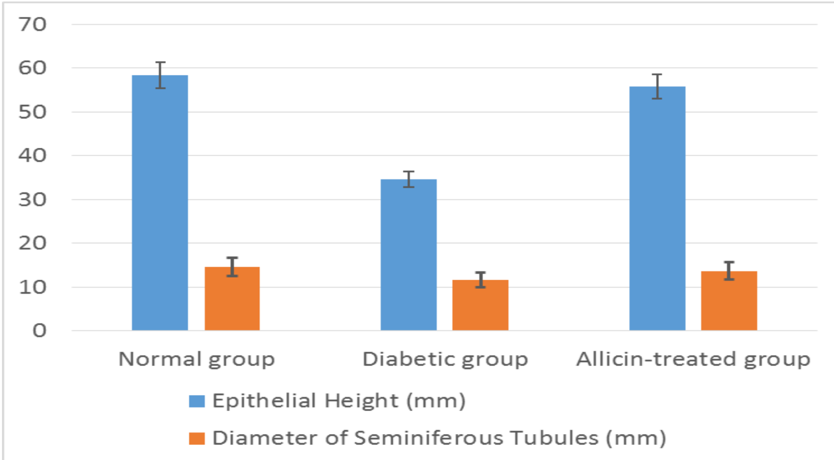

Figure (4). Representing the improvement of spermatogenesis in allicin-treated diabetic rats group vs rats with diabetes group

Discussion

Diabetes is considered as a serious global disease induction a state of chronic hyperglycemia, which is affecting several organs and system dysfunction, including reproductive system (Gaunay et al., 2013). Thus, many authors confirmed that male reproductive system dysfunction is evidently affected by diabetes mellitus which may result in partial infertility (Abdolahnejad et al., 2009; Tanaka et al., 2009; Sangameswaran et al., 2008). In addition, it is well established that morphologic changes in spermatogenesis is caused by diabetes due to apoptosis in spermatocytes and decreases the seminiferous tubules diameter which resulting in tubules atrophy (Cai et al., 2000 ; Guneli et al., 2008; Khaneshi, et al., 2013).

Correspondingly, present results were comparable to these previous studies that attributed to diabetes causes oxidative stress because of the increased of sugars level and lead to inter action with lipids and proteins, consequently increase the production of oxygen reactive species (ROS) that progressively served in the development of diabetic complications (Moody et al.,2008). So that In STZ-induced diabetes, Shahreari et al., (2010) showing that the alterations which are found in the seminiferous epithelium of diabetic animals that suggesting to the effect of ROS on Leydig cell function, testosterone level decreases.

On other hand, Agarwal and Said, (2004) referred that antioxidants act on protecting spermatogenic cells against damage caused by hyperglycemia. Since Several studies have demonstrated the importance of pharmacological activities of allicin as an antioxidant (Canizares et al., 2004; Macpherson et al., 2005; Reinhart et al., 2008). Overall, several studies have shown that antioxidant treatment improves glycemic index, reduces diabetic complications, and protects components from oxidative damage (Rahimi et al., 2005; Mohasseb et al., 2011).

The use of plants in the management of diabetes is well documented, which is primarily due to anti-hyperglycemic and/or oxygen radical scavenging of their various phytoconstituents through various mechanisms (Grover et al., 2002; Chan et al., 2012). Medicinal plants provide better alternatives as they are less toxic, easily available and affordable (Khan et al., 2012). Therefore, the current study stated that treatment with allicin amended testis tissue damage throughout preservation of seminiferous tubules, spermatogenic cells, and spermatids in rats with diabetes meanwhile diabetic rats exhibited extensive histopathological changes in architectural structure of testicular tissue. In this study, it has been demonstrated the apoptosis in spermatocytes and spermatids, in the diabetic rats which may result in impairment testis function, that was suggesting by one study indicated that it could be related to hyperglycemia induce high level of ROS production which it caused degeneration of cells (Shahreari et al., 2010). That was in accordance with recent study which revealed that Diabetes-mediated apoptosis contributes to the damage of targeted organs (Waisundara et al., 2008). It was also explained that hyperglycemia-induced apoptosis in testes, diabetic rats exhibited increased levels of oxidative stress detected by higher levels of ROS and lipid peroxidation, increased levels of apoptotic cells associated with upregulation of proapoptotic Bax and down regulation of antapoptotic Bcl-2 in testes (Long et al., 2015).

However, rats given allicin showing increase in number of healthy spermatocytes and spermatids that filled the lumen of seminiferous tubules that was because allicin has property in scavenge ROS as potent antioxidant. Hence, regarding that as a promising strategy that can target oxidative stress and modulate spermatogenesis for prevention diabetic complications affecting reproductive disorder.

Subsequently, allicin as traditional herbal medicine, which has been established to reduce oxidative stress and apoptosis to improve diabetic complications within testicular tissue. In this research, atrophy in seminiferous tubules in diabetic rats attributed to correlation in degree of reduction between diameter of seminiferous tubules and epithelial height was positive. Whereas in contrast, allicin- treated diabetic showing increases the diameter of seminiferous tubules and epithelial height that accompanies recent study about sesame effects which showed increasing of seminiferous tubules's diameter (Khaneshi et al., 2013). Moreover, that was clarified spermatogenesis improving in providing histological components protection and given positive effects on morphology that are necessary for successful spermatogenesis by allicin supplementation.

Conclusions

In conclusion, the results of this study indicated that allicin supplementation confers protection in morphology and function of the testis. Also it considered as a potential a nontoxic substance for the preservation of associated male reproductive fertility. Moreover, data showing allicin has ability to prevent and treat diabetes- male reproductive complication throughout reducing apoptosis of spermatocytes cells. Further work is needed to verify the action of allicin in clinical research.

REFERENCES

Abdolahnejad, A., Gol, A., & Dabiri, S. (2009). Garlic effects on reproductive complications of diabetes mellitus in male rats. Physiology and Pharmacology, 13(3), 297-307.

Agarwal, A., & Said, T. M. (2004). Carnitines and male infertility. Reproductive biomedicine online, 8(4), 376-384.

American Association of Diabetes Educators. (2002). Intensive diabetes management: implications of the DCCT and UKPDS. The Diabetes educator, 28(5), 735.

Ashraf, R., Khan, R. A., & Ashraf, I. (2011). Effects of garlic on blood glucose levels and HbA1c in patients with type 2 diabetes mellitus. Journal of Medicinal Plants Research, 5(13), 2922-2928.

Cai, L., Chen, S., Evans, T., Deng, D. X., Mukherjee, K., & Chakrabarti, S. (2000). Apoptotic germ-cell death and testicular damage in experimental diabetes: prevention by endothelin antagonism. Urological research, 28(5), 342-347.

Cañizares, P., Gracia, I., Gómez, L. A., García, A., de Argila, C. M., Boixeda, D., & de Rafael, L. (2004). Thermal Degradation of Allicin in Garlic Extracts and Its Implication on the Inhibition of the in-Vitro Growth of Helicobacter pylori. Biotechnology progress, 20(1), 32-37.

Chan, C. H., Ngoh, G. C., & Yusoff, R. (2012). A brief review on anti-diabetic plants: Global distribution, active ingredients, extraction techniques and acting mechanisms. Pharmacognosy reviews, 6(11), 22-28.

Chandra, A., Singh, R. K., & Tewari, L. (2004). Antioxidative potential of herbal hypoglycemic agents in diabetes–an overview. SFRR-India Bulletin, 3(1), 24-6.

Day, C. (1998). Traditional plant treatments for diabetes mellitus: pharmaceutical foods. British Journal of Nutrition, 80 (01), 5-6.

Eidi, A., Eidi, M., & Esmaeili, E. (2006). Antidiabetic effect of garlic (Allium sativum L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine, 13(9), 624-629.

Gaunay, G., Nagler, H. M., & Stember, D. S. (2013). Reproductive sequelae of diabetes in male patients. Endocrinology and metabolism clinics of North America, 42(4), 899-914.

Grover, J. K., Yadav, S., & Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. Journal of ethnopharmacology, 81(1), 81-100.

Guneli, E., Tugyan, K., Ozturk, H., Gumustekin, M., Cilaker, S., & Uysal, N. (2008). Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. European Surgical Research, 40(4), 354-360.

Kar, A., Choudhary, B. K., & Bandyopadhyay, N. G. (1999). Preliminary studies on the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. Journal of Ethnopharmacology, 64(2), 179-184.

Kesari, A. N., Kesari, S., Singh, S. K., Gupta, R. K., & Watal, G. (2007). Studies on the glycemic and lipidemic effect of Murraya koenigii in experimental animals. Journal of Ethnopharmacology, 112(2), 305-311.

Khan, V., Najmi, A. K., Akhtar, M., Aqil, M., Mujeeb, M., & Pillai, K. K. (2012). A pharmacological appraisal of medicinal plants with antidiabetic potential. Journal of Pharmacy and Bioallied sciences, 4(1), 27-42.

Khaneshi, F., Nasrolahi, O., Azizi, S., & Nejati, V. (2013). Sesame effects on testicular damage in streptozotocin-induced diabetes rats. Avicenna journal of phytomedicine, 3(4), 347-355.

Long, L., Wang, J., Lu, X., Xu, Y., Zheng, S., Luo, C., & Li, Y. (2015). Protective effects of scutellarin on type II diabetes mellitus-induced testicular damages related to reactive oxygen species/Bcl-2/Bax and reactive oxygen species/microcirculation/staving pathway in diabetic rat. Journal of diabetes research, 2015.

Lu, Y., He, Z., Shen, X., Xu, X., Fan, J., Wu, S., & Zhang, D. (2012). Cholesterol-lowering effect of allicin on hypercholesterolemic ICR mice. Oxidative medicine and cellular longevity, 2012.

Lucy, D., MD, A. S. Attele., DDS, Chun-Su, Y., MD, PhD. (2002). Alternative Therapies for Type 2 Diabetes. Alternative Medicine Review, 7(1), 45-58.

Macpherson, L. J., Geierstanger, B. H., Viswanath, V., Bandell, M., Eid, S. R., Hwang, S., & Patapoutian, A. (2005). The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Current Biology, 15(10), 929-934.

Mariee, A. D., Abd-Allah, G. M., & El-Yamany, M. F. (2009). Renal oxidative stress and nitric oxide production in streptozotocin-induced diabetic nephropathy in rats: the possible modulatory effects of garlic (Allium sativum L.). Biotechnology and applied biochemistry, 52(3), 227-232.

Metwally, N. S., Mohamed, A. M., & ELSharabasy, F. S. (2012). Chemical constituents of the Egyptian Plant Anabasis articulata (Forssk) Moq and its antidiabetic effects on rats with streptozotocin-induced diabetic hepatopathy. Journal of Applied Pharmaceutical Science, 2(4), 54-65.

Mohasseb, M., Ebied, S., Yehia, M. A., & Hussein, N. (2011). Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats. Journal of physiology and biochemistry, 67(2), 185-194.

Moody, B., Heydari, G., Mahmoud Zadeh, C., & Harati, M. (2008). Study biochemistry and histology sodium tungstate protective effect on oxidative stress induced by streptozocinin the pancreasof diabeticrats. Anatomy, 22, 279-292.

Mustafa, S. S., Eid, N. I., Jafri, S. A., El-Latif, H. A. A., & Ahmed, H. M. (2007). Insulinotropic effect of aqueous ginger extract and aqueous garlic extract on the isolated perfused pancreas of streptozotocin induced diabetic rats. Pakistan Journal of Zoology, 39(5), 279.

Navarro-Casado, L., Juncos-Tobarra, M. A., Chafer-Rudilla, M., Onzoño, L. Í., Blazquez-Cabrera, J. A., & Miralles-Garcia, J. M. (2010). Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. Journal of andrology, 31(6), 584-592.

Rahimi, R., Nikfar, S., Larijani, B., & Abdollahi, M. (2005). A review on the role of antioxidants in the management of diabetes and its complications. Biomedicine & Pharmacotherapy, 59(7), 365-373.

Reinhart, K. M., Coleman, C. I., Teevan, C., Vachhani, P., & White, C. M. (2008). Effects of garlic on blood pressure in patients with and without systolic hypertension: a meta-analysis. Annals of Pharmacotherapy, 42(12), 1766-1771.

Reinhart, K. M., Coleman, C. I., Teevan, C., Vachhani, P., & White, C. M. (2008). Effects of garlic on blood pressure in patients with and without systolic hypertension: a meta-analysis. Annals of Pharmacotherapy, 42(12), 1766-1771.

Roman-Ramos, R., Flores-Saenz, J. L., & Alarcon-Aguilar, F. J. (1995). Anti-hyperglycemic effect of some edible plants. Journal of ethnopharmacology, 48(1), 25-32.

Sangameswaran, B., & Jayakar, B. (2008). Anti-diabetic, anti-hyperlipidemic and spermatogenic effects of Amaranthus spinosus Linn. On streptozotocin-induced diabetic rats. Journal of natural medicines, 62(1), 79-82.

Sicree R, Shaw JE, Zimmet PZ. (2003). Diabetes Atlas. 2nd ed. Brussels: International Diabetes Federation. The global burden of diabetes; pp. 15–71.

Tanaka, M., Nakaya, S., Kumai, T., Watanabe, M., Matsumoto, N., & Kobayashi, S. (2009). Impaired testicular function in rats with diet-induced hypercholesterolemia and/or streptozotocin-induced diabetes mellitus. Endocrine research.

Thomson, M., Al-Amin, Z. M., Al-Qattan, K. K., Shaban, L. H., & Ali, M. (2007). Anti-diabetic and hypolipidaemic properties of garlic (Allium sativum) in streptozotocin-induced diabetic rats. Int J Diabetes & Metabolism, 15, 108-115.

Waisundara, V. Y., Hsu, A., Huang, D., & Tan, B. K. H. (2008). Scutellaria baicalensis enhances the anti-diabetic activity of metformin in streptozotocin-induced diabetic Wistar rats. The American journal of Chinese medicine, 36(03), 517-540.