*Nig. J. Physiol. Sci. 26(June 2011) 103 – 107*
www.njps.physocnigeria.org

Nig. J. Physiol. Sci.

# Garlic and vitamin E provides antioxidant defence in tissues of female rats treated with nicotine

**Iranloye B.O., Oludare G.O.**
*Department of Physiology, College of Medicine, University of Lagos, Nigeria.*

**Summary:** Nicotine is known to induce oxidative stress in rat tissues and the antioxidant properties of garlic have been reported. This study was designed to determine if the peroxidative damage caused by nicotine administration can be effectively prevented with garlic juice, and vitamin E, a known antioxidant.Four groups of six rats each were divided into: Group I: (control) received 0.2ml of 0.9% normal saline, group II (received nicotine 0.6mg/kg b.w subcutaneously), group III (received nicotine 0.6mg/kg b.w + garlic juice 100mg/kg b.w orally), and group IV (received nicotine 0.6mg/kg b.w + Vitamin E 100mg/kg b.w orally). All animals were treated for 21 days. The pituitary gland, ovary, uterus, heart, liver and kidney of the animals were harvested, weighed and homogenized. Malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH) were then measured.Concentration of MDA was significantly increased in tissues of nicotine treated rats when compared with the control. In group III and IV, MDA levels were significantly reduced when compared with nicotine group. The activities of SOD and GSH significantly decreased in group II (nicotine only) rat tissues, while it was significantly increased in group III and IV rat tissues. The study showed that garlic juice extract (100mg/kg b.w) and vitamin E (100mg/kg b.w) administration prevented oxidative damage in rat tissues treated with nicotine. The study also showed that vitamin E has a more potent antioxidant activity than garlic juice in preventing nicotine induced oxidative damage in rat.

**Keywords*:*** Nicotine, Vitamin E, Garlic, antioxidant



**Address for correspondence: bolasunkanmi45@yahoo.com*

*Manuscript Accepted: February, 2011*

## INTRODUCTION

The formation of reactive oxygen species (ROS) in cells leads to the formations of radicals in metabolic processes which causes damages to many molecules in cells, including membrane lipids, proteins and nucleic acids (Ilker et al, 2004). These harmful effects are controlled by antioxidant defense system in cells which include the enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose -6- phosphate dehydrogenase (Erat et al, 2007).

All over the world the use of complementary and alternative medicine is escalating rapidly. Out of pocket expenditure for herbal therapies are estimated at more than 5 billion dollars per year in the united states alone (Ronald et al, 2001). Garlic (Allium Sativum) is clearly one of the most popular herbal remedy used worldwide today for the treatment of various ailments. Previous animal studies has suggested that garlic has potential antilipidemic, antioxidant, antihypertensive, antiglycemic, anti-thrombotic and antitherogenic properties (Ronald et al, 2001, Borek et al 2001, Wang et al 1998).

Many drugs and chemicals can increase the rate of ROS/free radicals formation in specific organs of the body. Nicotine a major toxic component of cigarette smoke has been reported to affect trace element relationship between tissues as well as components of the free radical defense system (Dubick & Keen 1991, Husain et al 2001). Nicotine has also been shown to induce tissue damage in the uterus, ovary and the pituitary gland which are involved in the reproductive life of the female and may explain the adverse reproductive outcome such as infertility, subfecundity, menstrual disorders and prolonged estrous seen in female smokers/nicotine treated rats. (Iranloye & Bolarinwa 2009, Iranloye & Bolarinwa 2007, Holloway et al, 2006, United States Department of Health and Human Services 2001)

Since nicotine has been reported as having oxidant effect on tissues (Erat et al, 2007) while garlic as well

as vitamin E are believed to posses antioxidant properties( Ilker et al 2004). This study was designed to determine the effect of garlic and vitamin E on nicotine induced tissue damage in female rats.

## MATERIALS AND METHODS

### Animals

Twenty four (24) female rats weighing between 140-160g were used in this study. The rats were fed with normal rat pellets and water ad libithum. They were randomly divided into four (4) groups of six animals each as follows:

Group I (Control) received 0.9% normal saline for 21 days.

Group II Nicotine- treated (NT) received nicotine 0.6mg/kg body weight subcutaneously for 21 days

Group III Nicotine + Garlic treated (NGT) received nicotine 0.6mg/kg b.w subcutaneously and garlic 100mg/kg b.w orally for 21 days.

Group IV Nicotine + Vitamin E treated (NVET) received nicotine 0.6mg/kg b.w subcutaneously and Vitamin E 100mg/kg b.w orally for 21 days.

All animals were sacrificed at the end of 21 days by cervical dislocation. The organs – kidney, liver, heart, pituitary gland, uterus and ovary were removed cleared of fats, weighed, homogenized and stored in plain sample bottles for analysis of MDA, reduced glutathione and superoxide dismuthase.

### Preparation of Garlic Juice And Nicotine

Garlic juice was prepared using the method of Ibu *et al* (2005). The fresh garlic cloves were peeled on crushed ice, and 50g of garlic was homogenized in 75ml of cold normal saline in the presence of some crushed ice. The filtered homogenized mixture was then centrifuged at 2000xg for 10 minutes and the clear supernant was made up to 500ml with normal saline to yield a concentration of 100mg/ml of garlic. The prepared garlic extract was stored at -20$^{o}$c until use.

### Lipid Peroxidation Assay

The level of lipid peroxidation was estimated as the concentration of thiobabituric acid reactive product malondialdehyde (MDA) as described by Ohkawa et al, (1979). 1ml of the tissue homogenate was thoroughly mixed with 2ml of TCA-TBA-HCl solution and heated for 15 minutes in a water bath. After cooling, the precipitate is removed by centrifugation and the absorbance measured at 523nm using a sphectrophotometer.

### Reduced Glutathione

Glutathione (GSH- Reduced) was also determined using 5,5 Dithro-bis 2- Nitrobenzoic acid (DNTB) and Tris-EDTA buffer as described by Tappel (1978). 100ul of the sample was added to 1ml of 0.2ml Tris-

EDTA buffer (pH 8.2) followed by 0.9ml of 20mM EDTA (pH 4.7) and 20ul DNTB. The sample was incubated at room temperature for 30 minutes. The mixture was centrifuged and absorbance of the supernant read at 412nm.

### Superoxide Dismuthase

Superoxide dismuthase activity was assayed as described by Sum et al (1978). The reaction was carried out in 0.5m sodium carbonate buffer pH 10.2 and was initiated by the addition of 3 X $10^{-4}$ epinephrine in 0.005N HCl. The absorbance was read at 320nm.

### Statistical Analysis

Data analysis was carried out by one way- analysis of variance (ANOVA) supported by the Newman- Keuls test when pair wise comparism was done between the groups. Results were presented as means ± SEM, and the differences were considered significant at $P< 0.05$. Statistical analysis was performed with Graphic Pad Prism.

## RESULTS

### Organ Weights

The organ weights of the pituitary and the heart were significantly increased ($P< 0.05$) in NT rats compared with control. NGT and NVET also significantly decreased the weight of the heart when compared with the NT group. No significant difference was observed in the weight of other organs studied.

### Lipid Peroxidation

The concentration of MDA were increased significantly ($P < 0.05$) in the pituitary gland, ovary, heart, liver and kidney of NT rats compared with the control rats. NGT rats showed significantly decreased ($P < 0.05$) MDA levels in the pituitary, ovary, heart and the liver when compared with NT rats. NVET rats showed significantly decreased ($P < 0.05$) MDA levels in the pituitary, ovary, heart, liver and kidney when compared with NT rats. Comparing the effect of the two antioxidants, NGT significantly reduced the MDA levels in the ovary than NVET.

### Superoxide Dismutase

SOD was significantly decreased ($P < 0.05$) in the pituitary, ovary, uterus, heart, liver and kidney of NT rats when compared with control. NGT showed increased activity of superoxide dismutase in all the tissues studied when compared with NT rats. NVET also increased the activity of SOD in all the organs studied when compared with NT rats. NVET increased SOD activity significantly in the pituitary,

uterus, heart and the liver when compared with NGT rats.

**Table 1.**
Mean organ weights (g) of control and animals treated with nicotine, garlic and vit E

| | Group I (Control) | Group II (NT) | Group III (NGT) | Group IV (NVET) |
|---|---|---|---|---|
| Pituitary | 0.032±0.002 | 0.056±0.01* | 0.044±0.01 | 0.045±0.002 |
| Ovary | 0.14±0.01 | 0.15±0.02 | 0.14±0.03 | 0.22±0.04 |
| Uterus | 0.113±0.01 | 0.112±0.02 | 0.109±0.02 | 0.125±0.01 |
| Heart | 0.52±0.02 | 0.69±0.06* | 0.57±0.02 [a] | 0.55±0.01 [a] |
| Liver | 5.35±0.49 | 5.0±0.5 | 5.11±0.39 | 5.17±0.01 |
| Kidney | 0.5±0.04 | 0.6±0.05 | 0.52±0.04 | 0.58±0.06 |

* Values are mean ± SEM of six rats. P< 0.05, vs. control, [a] vs. NT,

**Table 2.**
Mean malonidialdehyde (MDA) concentration (nmol/ml) in the organs of control animals and animals treated with nicotine, garlic and vitamin E.

| | Group I (Control) | Group II (NT) | Group III (NGT) | Group IV (NVET) |
|---|---|---|---|---|
| Pituitary | 16.3 ± 2.09 | 22.4 ± 1.63* | 16.4 ± 0.87[a] | 16 ± 1.4[a] |
| Ovary | 27.5 ± 1.71 | 41.7 ± 1.92* | 26.5 ± 1.14[a] | 34.8 ± 1.17[a,b] |
| Uterus | 11.2 ± 0.81 | 13.4 ± 2.26 | 10.9 ± 1.22 | 11.65 ± 1.21 |
| Heart | 3.83 ± 0.81 | 7.6 ± 0.84* | 2.7 ± 1.16[a] | 3.9 ± 1.18[a] |
| Liver | 6.58 ± 1.19 | 13.45 ± 1.92* | 7.37 ± 1.81[a] | 6.2 ± 0.92[a] |
| Kidney | 4.43 ± 0.12 | 8.12 ± 1.47* | 5.06 ± 0.83[a] | 3.45±0.32[a] |

* Values are mean ± SEM of six rats. P< 0.05, vs. control, [a] vs. NT, [b] vs. NT

**Table 3.**
Mean values of superoxide dismutase (mg/ml) activity in the organs of control animals and animals treated with nicotine, garlic and vitamin E.

| | Group I (Control) | Group II (NT) | Group III (NGT) | Group IV (NVET) |
|---|---|---|---|---|
| Pituitary | 227.3 ± 1.74 | 112.8 ± 2.07* | 150.7 ± 0.82[a] | 170 ± 1.11[a,b] |
| Ovary | 119.7 ± 1.19 | 73.2 ± 0.93* | 105.2 ± 2.19[a] | 106.7 ± 3.05[a] |
| Uterus | 120.2 ± 1.42 | 93.1 ± 1.54* | 102.8 ± 1.24[a] | 107.6 ± 1.42[a,b] |
| Heart | 262.9 ± 1.38 | 133.3 ± 1.07* | 213.9 ± 2.85[a] | 230.5 ± 1.64 [a,b] |
| Liver | 200.5 ± 1.52 | 134 ± 1.85* | 213.8 ± 1.72[a] | 230.6 ± 1.24 [a,b] |
| Kidney | 225.2 ± 1.57 | 120.2 ± 1.37* | 186.3 ± 1.16[a] | 179.2 ± 3.5 [a,b] |

* Values are mean ± SEM of six rats. P< 0.05, vs. control, [a] vs. NT, [b] vs. NT

**Table 4.**
Mean values of reduced glutathione (umol/ml) activity in the organs of control animals and animals treated with nicotine, garlic and vitamin E.

| | Group I (Control) | Group II (NT) | Group III (NGT) | Group IV (NVET) |
|---|---|---|---|---|
| Pituitary | 0.53 ± 0.03 | 0.21 ± 0.07* | 0.84 ± 0.02[a] | 0.89 ± 0.02[a] |
| Ovary | 0.53 ± 0.01 | 0.28 ± 0.008* | 0.82 ± 0.02[a] | 0.76 ± 0.008[a,b] |
| Uterus | 0.18 ± 0.01 | 0.16 ± 0.01 | 0.14 ± 0.01 | 0.17 ± 0.01 |
| Heart | 0.34 ± 0.02 | 0.17 ± 0.01* | 0.34 ± 0.01[a] | 0.37 ± 0.02[a] |
| Liver | 0.29 ± 0.01 | 0.16 ± 0.008* | 0.35 ±0.01[a] | 0.51 ± 0.02[a,b] |
| Kidney | 0.3 ± 0.008 | 0.13 ± 0.008* | 0.14 ± 0.008[a] | 0.43 ± 0.02[a] |

* Values are mean ± SEM of six rats. P< 0.05, vs. control, [a] vs. NT, [b] vs. NT

**Reduced Glutathione**

From table 4, significant decrease in reduced glutathione at (P < 0.05) was observed in the pituitary, ovary, heart, liver and kidney of NT rats when compared with the control. NGT showed significant increases in the levels of reduced glutathione of the pituitary, ovary, heart, liver and kidney when compared with the NT rats. NVET also significantly increased the level of reduced glutathione in the pituitary, ovary, heart, liver and kidney when compared with the NT rats. When the effects of both antioxidants were compared, NGT had a significant increase (P < 0.05) in GSH levels of the ovary than NVET. On the other hand, NVET had a significant increase in GSH levels of the liver than NGT.

## DISCUSSION

This study confirms earlier reports of the toxicity of nicotine on some visceral organs e.g pituitary, heart, kidney, liver, ovary, brain, adrenal gland and uterus

(Iranloye and Bolarinwa 2009). Significant increases in the weights of the pituitary gland and the heart in nicotine treated rats were observed in this study. Report have it that, increase or decrease in either absolute or relative weight of an organ after administering a chemical or drug is an indicator of the toxic effects of that chemical (Simons et al, 1995, Nwanjo et al, 2007). Thus, confirming the toxicity of nicotine in animals.

The observed lipid peroxidation in the liver and reduced oxidative enzymes by nicotine affirms the ability of nicotine to induce tissue damage. In this study, garlic and vitamin E proved to be efficient antioxidant by enhancing the levels of antioxidant enzymes reduced by nicotine. Erat et al, (2007) gave a similar report in which vitamin E prevented the inhibition of glutathione reductase by nicotine in the liver, testicle, heart, stomach and kidney.

Lipid peroxidation was induced by nicotine in the pituitary and the ovary while there was no significant effect on peroxidation in the uterus. This suggests that nicotine action on the pituitary- ovarian axis might as well be via lipid peroxidation in addition to its antiestrogenic effect earlier reported by Blackburn et al (1993). Necrosis and degeneration of cells in the anterior region of the pituitary and follicular degeneration has earlier been reported in the pituitary and ovary of rats (Iranloye and Bolarinwa 2009). This might be due to oxidative stress which has been associated with cell degeneration in dopaminergic cells of mice (McCormack et al, 2005). The lipid peroxidation in nicotine treated rats was accompanied by depletion of antioxidant enzyme SOD and GSH. Garlic, provided antioxidant activity on the rat tissues by increasing the levels of SOD and GSH thus reducing MDA in the tissues of the pituitary and ovary. This characteristic of antioxidants was also exhibited by vitamin E in the tissues. Gumustekin et al (2003) have reported that nicotine inhibited the activity of glutathione peroxidase of the brain while Vitamin E abolishes the effect. Similar report by Suleyman et al (2002) showed that nicotine inhibited the activities of glutathione peroxidase and SOD of erythrocytes, while vitamin E prevented these effects.

Lipid peroxidation and depletion of antioxidant enzymes were also observed in the heart and kidney of rats treated with nicotine in this study. Evidence by earlier works suggests that ROS may play an important role in the pathogenesis of myocardial infarction (Loeper et al, 1991, Pasupathy et al 2009). This study showed that garlic and vitamin E enhanced antioxidant enzymes SOD and GSH and reduced the MDA levels increased by nicotine. The exact link between nicotine and kidney disease is still not well understood, studies has shown that those who use nicotine seem to be at a higher risk for proteinuria (Freidman and Fadem 2010). The observed lipid peroxidation in this study might suggest a possible mechanism of action of nicotine on the kidney. Garlic juice and Vitamin E treatment in this study reversed these effects of nicotine on the kidney.

This study has shown that garlic and vitamin E treatment prevented the increase in MDA, probably in part by scavenging the very reactive hydroxyl and peroxyl radicals and enhanced the production of antioxidant enzyme depleted by nicotine. This is in line with previous work by Erat et al, (2007), in which vitamin E prevented the inhibition of glutathione reductase in the liver, kidney, testicle, heart and stomach of nicotine treated rats. While Sener et al, (2005) reported that garlic reversed MDA increases in nicotine treated rat tissues (kidney, urinary bladder, aorta and heart).Vitamin E activity on SOD and GSH of the tissues studied was more pronounced in the pituitary, uterus, heart and liver while the activity of garlic was more pronounced in the kidney and ovary. This suggests that vitamin E has more pronounced effect than garlic at the same concentration of 100mg/kg b.w. In conclusion, the antioxidant effect of garlic on the tissues reported in this study suggests that its consumption may reduce the oxidative stress induced by nicotine. Thus garlic and vitamin E may be beneficial in oxidative stress diseases induced by nicotine such as, atherosclerosis, myocardial infarction, menstrual disorders among others.

## REFERENCES

Blackburn, C.W., Peterson, C.A., Hales, H.A., Carrell, D.T., Jones, K.E., Urry, R.L., Peterson, C.M., (1994). Nicotine, but not cotinine, has direct toxic effect on the ovarian function in the immature gonadotropin- stimulated rat. Reprod. Toxicol. 8(4): 325-331.

Borek, C (2001). Antioxidant effect of aged garlic extract. J. Nutr. 131:1010s-1015s.

Department of Health and Human Servies. 2001. Women and smoking; A report of the surgeon general. Washington DC: US Department of health and Human services, Public Health service, office of the surgeon general.

Dubick M.A., Keen C.L (1991) Influence of nicotine on tissue trace elements concentration and tissue antioxidants. Boil. Trace. Elem. Res. 31, 97-103.

Friedman A.N, Fadem S.Z (2010). Reassesment of albumin as a nutritional marker in kidney disease. J Am. Soc. Nephrol 21: 223-230.

Gumustekin, K, Altinkayanak, K, Timur, H, Taysi S, Oztasan N, Polat M.F, Akcay, F, Suletman H, Dane, S, Gul M. (2003) Vitamin E but not *Hippophea rhamnoides* L. prevented nicotine

induced oxidative stress in rat brain. Human Exp Toxicol. 22, 425-431.

Holloway A.C., Kellenberger, L.D., Petrick J.J. (2006). Fetal and neonatal exposure to nicotine disrupts ovarian function and fertility in adult female rats. Endocrine vol 30 No 2: 213-226

Husain, K., Scott, B.R., Reddy, S.K., Somani, S.M., (2001) Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. Alcohol 25, 89-97.

Ibu, J.O, Dickson, A.J, Eno, A.E (2005). Effect of an aqueous extract of allium sativum linn on the interstinal motility of the rabbit. Nig Journal of health and biomedical sciences vol 4, No 1, 40-45.

Ilker, D., Bilal, A., Yusuf, A., Erdinc, D., Aslihan, A., Cetin, E., Dervis, O., (2004). Effects of garlic extract consumption on plasma and erythrocyte antioxidant parameters in atherosclerotic patients. Life sciences 75: 1959-1966.

Iranloye, B.O., Bolarinwa, A.F, (2009).Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats. Nigerian Journal of Physiological Sciences 24 (1): 7 – 12

Iranloye, B.O., Bolarinwa, A.F, (2007). Effect of nicotine administration on estrous cycle in female albino rats. Nigeria Journal of Health and Biomedical Sciences Vol 6 (2) : pp. 21-25.

Loeper, J., Goy, J.and Rozenstajin, L. (1991). Lipid peroxidation and protective enzymes during myocardial infarction. Clin Chim Acta, 196: 119-126.

Erat M., Mehmet. C., Kenan. G., Mustafa. G., (2007) Effects of nicotine and vitamin E on glutathione reductase activity in some rat tissues in vivo and in vitro. European Journal of Pharmacology 554: 92-97.

McCormack AL, Atienza JG, Johnson LC, Andersen JK, Vu S, Di Monte D.A (2005) Role of oxidative stress in paraquat-induced doparminergic cell degeneration. J Nuerochem 93(4): 1030-7

Nwanjo, H.U., Iroagba, I.I., Nnatuanya,I.I and Eze, N.A (2007). Antifertility activity of dihydroartemisin in male albino rats. Journal of endocrinology vol 4 number 1.

Ohkawa H, Oshishi n and Yagi K (1979) Assay of lipid peroxides in animals by thiobarbituric acid reactives. Anal. Biochem, 95, 351-358.

Pasupathi P, RaoY.Y, Farook J, Saravanan G, Bakthavathsalam G. (2009) Effect of Cigarette Smoking on Lipids and Oxidative Stress Biomarkers in Patients with Acute Myocardial Infarction. Res. J. Medicine & Med. Sci., 4(1): 151-159, 2009

Ronald, T.A., Cynthia, D.M., Gilbert, R., Christopher, D.G., Laura, M., & Valerie A.L (2001). Garlic shows promise for improving some cardiovascular risk factors. Arch Intern Med 161: 813-824.

Sener, G, Sehirli, A.O, Imipc¸Y, Cetinel, S, Cikler, E., Gedik, N (2005) Chronic Nicotine Toxicity is Prevented by Aqueous Garlic Extract. Plant Foods for Human Nutrition 60: 77–86.

Sum, M.,& Zigma, S., (1978). An improved spectrophotomeric assay for superoxide dismutase based on epinephrine autooxidation. Analyst Biochem 90: 81-89.

Simons, I.E,Yang RSH, Berman, R, (1995). Evaluations of nephrotoxicity of complex mixtures containing organics and metals: advantages and disadvantages of real-world complex mixtures. Environ. Health Perspect. 103: 67-71

Suleyman, H, Gumustekin, K, Tasyi, S, Keles, S, Oztasan, N, Aktas, O, Altinkaynak, K, Timur, H, Akcay, F, Akar, S, Dane, S, Gul, M (2002). Beneficial effects of *Hippophae rhamnoides* L. on nicotine induced oxidative stress in rat blood compared with vitamin E. Biol. Pharm. Bull. 25, 1133-1136.

Tappel, A.L (1978). Glutathione peroxidase and hydroperoxides. Meth. Enzyme 52. 506-513.

Wang B.H, Katherine A.Z., Khalid, R., and David, B., (1998). Protective effects of aged garlic extract against bromobenzene toxicity to precision cut rat liver slices. Toxicology 126: 213-222.