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Evaluation of Consumption of Mixed Diet of Garlic, Garden Egg and Groundnut on the Cytoarchitecture of the Spleen of Anaemic Wistar Rats

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Abstract: ***Twenty five induced anaemic wistar rats of both sexes weighing 160-200g were used in this study to evaluate the effectiveness of consumption of mixed diet of garlic, garden egg and groundnut on the cytoarchitecture of the spleen. The animals were divided into five groups labelled 1, 2, 3, 4 and 5. Group 1 served as the non-anaemic control and was fed with normal rat chow. Groups 2, 3, 4 and 5 the experimental, were induced with anaemia by oral administration of phenylhydrazine (PHZ) given at 50mg/kgBW. Group 2 served as anaemic control, fed with normal rat chow; Group 3 were fed with 20g (75%ww) of garlic, garden egg and groundnut in the ratio of 1:1:1 with normal rat chow; Group 4 fed with 10g of garden egg (50%ww) in the ratio 1:1 with normal rat chow and Group 5 fed with 10g of groundnut (50%ww) in the ratio 1:1 with normal rat chow. Animals were sacrificed at the end of fourteen days of dietary feeding using chloroform inhalation method. The spleen were dissected out and fixed in 10% formal saline. Cytoarchitecture of spleen were observed in groups 1, 3 4 and 5 rats to be normal.. Sections from group 2 anaemic control rats, showed distortion of splenic white pulp with degenerated splenic nodules and diffused red pulp. Thus, the results from this study showed that the mixed diet of garlic, garden egg and groundnut have a protective effect on the cytoarchitecture of the spleen of induced anaemic wistar rats.***

Keywords: anaemia, garlic, garden egg, groundnut, phenylhydrazine, diet, spleen

1. Introduction

Worldwide, varieties of plants are consumed both for dietary and therapeutic purposes. Of such plants commonly consumed especially in Nigeria include garlic (Allium sativum), garden egg (Solanum melongena) and groundnut (Arachis hypogeae). A broad range of therapeutic responses to garlic have been reported, including decreases in blood pressure, blood lipid and glucose concentrations, and the risk of atherosclerosis as well as antimicrobial, anticancer and hepatoprotective effects [1-3]. Garden egg has been shown to be beneficial to diabetic patients, used as a remedy for liver complains [4], beneficial to patients suffering from glaucoma and convergence insufficidency [5] and diseases associated with hyperlipidemia such as arteriosclerosis. Garden egg also produces antipyretic (dose-dependent) and analgesic effect and reduces blood level of total and LDL cholesterol [6]. Groundnut commonly referred to peanuts is a widely consumed legume. Several studies have demonstrated its health benefits including cardio-protective benefits [7-8], reduced risk of stroke [9], anticancer [10-11], and reduced risks of Alzheimer and weight gain [12-14].

The spleen is comprised of two histologically and functionally distinct organs; one is a phagocytic organ, the red pulp, and the other is an immune organ, the white pulp [15]. Considering its central role in the sequestration and destruction of drug-damaged erythrocytes, the spleen is expected to be affected in response to hemolysis. Phenylhydrazine and its hydrochloride derivatives have been extensively used experimentally to study oxidative haemolysis. Previously, these compounds were used for the treatment of Polycythemia [16]. It has been documented that a healthy diet may improve or maintain optimal health [17]. Thus, this study was carried out to investigate the possible protective effect of consumption of mixed diet of garlic, garden egg and groundnut on the histology of the spleen of induced anaemic wistar rats.

2. Materials and Methods

2.1 Breeding of animals

Twenty five adult Wistar rats of both sexes weighing between 160-200g were used. They were obtained from the animal house of Department of Physiology, University of Calabar and kept in the animal house of the Department of Human Anatomy for a period of two weeks under standard conditions of temperature 27˚C - 30˚C, photo period of 12-hour natural light cycle and 12-hour dark to acclimatize. They were fed with pelleted chick mash manufactured by Agro Feed Mill Nigeria Ltd and drinking water given ad libitum. After the acclimatization period, they were randomly divided into five groups of five rats each; two controls and three experimental groups.

2.2 Preparation of the diet

Garlic, garden egg and groundnut were bought from Watt Market in Calabar, Cross River State, Nigeria and were identified by the botanist in the botanical garden of the University of Calabar. The plants were washed with water to remove impurities and samples certified as spoiled or attacked by infections were removed. Garlic was defoliated then minced while the garden egg was chopped. The garlic, garden egg and groundnuts were dried in carbolite moisture extraction drying oven (Grant Instruments, Cambridge England) at 50˚c. The groundnut was dried for an hour while the garden egg and garlic were dried for three hours. The now dried samples were blended into coarse powdered form and kept in glass containers with plastic cover to keep them airtight.

2.3 Induction of anaemia

Anaemia was induced by oral administration of phenylhydrazine (PHZ) given at 50mg/kgBW for the first two days and then at an interval of three days as maintenance dose. Anaemia was confirmed by test of haemoglobin (Hb) level using haemoglobemeter (Hemocue Hb 201^{+}, Ängelholm, Sweden) using blood samples collected by nipping of rat's tail. Pheylhydrazine is a known haemolysing drug [19].

2.4 Experimental protocol

The rats were divided into five groups of five rats each and placed on a two week feeding regimen as follows:

Group 1 - Negative control, non-anaemic and fed with normal rat chow;
Group 2 - Positive control, anaemic rats and fed with normal rat chow;
Group 3 – Pnaemic rats, fed with 20g (75%ww) of garlic, garden egg and groundnut in the ratio of 1:1:1 with rat chow;
Group 4 – Pnaemic rats, fed with 10g of garden egg (50%ww) in the ratio 1:1 with rat chow
Group 5 – Pnaemic rats, fed with 10g of groundnut (50%ww) in the ratio 1:1 with rat chow.

2.5 Termination of the experiment

The rats were sacrificed at the end of fourteen days of dietary feeding using chloroform inhalation method. The spleen were dissected out and fixed in 10% formal saline for 48hours, processed and sections stained using Hematoxylin and Eosin staining method.

3. Results

Splenic tissue obtained from animals in normal control group shows normal histology of the spleen. Splenic white pulp show well defined splenic nodules. The red pulp was also prominent. Sections from rats in anaemic control group shows splenic white pulp with degenerated splenic nodules and diffused red pulp. Test Groups 3, 4 and 5 showed normal cyto architecture of the spleen as seen in the normal control group.

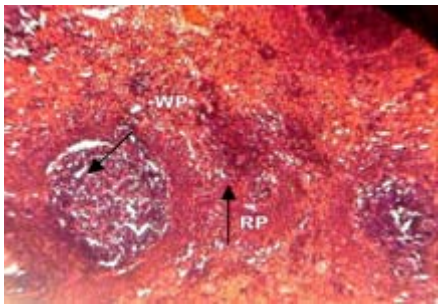

Figure 1: Photomicrograph of spleen of negative control that received normal rat chow showing normal splenic architecture, made up of the white pulp (WP) with well defined splenic nodules and red pulp (RP) (H & E X 100).

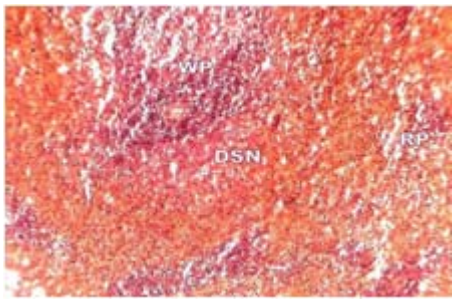

Figure 2: Photomicrograph of spleen of positive anaemic control that received normal rat chow showing distortion of the white pulp (WP) with degenerated splenic nodule (DSN) and diffused red pulp (RP). (H & E X 100).

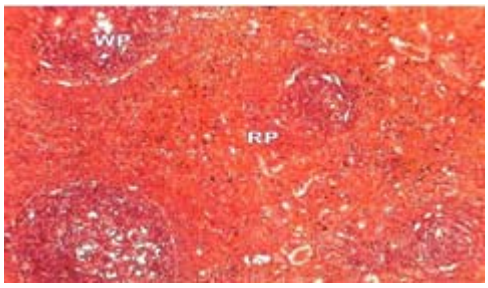

Figure 3: Photomicrograph of anaemic rats fed with 20g (75%ww) of garlic, garden egg and groundnut in the ratio of 1:1:1 with normal rat chow showing normal splenic architecture (H & E X 100).

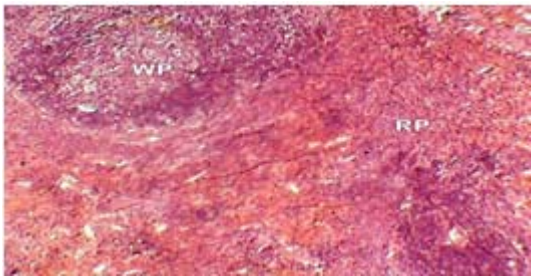

Figure 4: Photomicrograph of anaemic rats fed with 10g of garden egg (50%ww) in the ratio 1:1 with normal rat chow showing normal splenic appearance (H & E X 100).

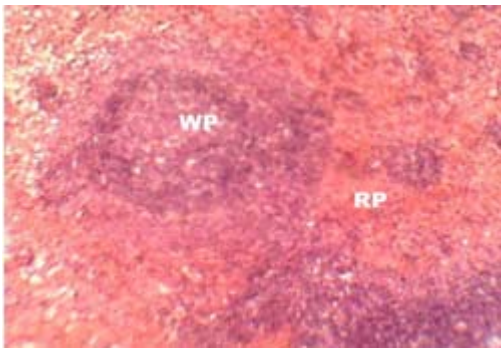

Figure 5: Photomicrograph of anaemic rats fed with 10g of groundnut (50%ww) in the ratio 1:1 with normal rat chow showing normal splenic appearance (H & E X 100)

4. Discussion

Considering its central role in the sequestration and destruction of drug-damaged erythrocytes, the spleen is expected to be affected in response to haemolysis [18]. Phenylhydrazine is a known haemolysing drug [19]. The

histological features of the spleen administered with phenylhydrazine alone (positive control) showed distorted white pulp and red pulp. Altered splenic cytoarchitecture after administration of phenylhydrazine was also observed in studies carried out by [20] and [21].

Experimental animal studies have demonstrated that the splenic arterial system is necessary for optimal control of infection [22], thus splenic filtering function is important in maintaining normal body morphology and functioning. In this study, it was observed that consumption of combined diet of garlic, garden egg and groundnut, diet of garden egg or groundnut could restore back or preserved splenic cytoarchitecture. This could be attributed to the antioxidant properties present in garlic, garden egg and groundnut. Research evidences points to the fact that antioxidants such as polyphenols, flavonoids, DADs and vitamins A, C and E have the capacity to perfectly regulate the production of reactive oxygen species and foods of high antioxidant activity have been encouraged for consumption.

5. Conclusion

Based on the current research it be said that phenylhydrazine induces splenic cytoarchitectural distortion which was reversible after consumption of garlic, garden egg and groundnut. This further supports the nutritional and therapeutic health benefits of usage of these plants.

References

[1] KC. Agarwal. Therapeutic actions of garlic constituents. Medical Research and Review, 1, 111-124, 1996.
[2] KT. Augusti, Therapeutic values of onion (*Allium cepa L.*) and galic (*Allium sativum L.).* Indian Journal of Experimental Biology, 34: 634 – 640, 1996.
[3] JH. Campbell, JL. Efendy, WJ. Smith, GR. Campbell. Molecular basis by which garlic suppresses artherosclerosis. Journal of Nutrition, 131: 1006S – 1009S, 2001.
[4] DK. Salunkhe, SS. Kadam. Handbook of Vegetable Science and Technology. Marcel Dekker Inc. New York. 721,1998.
[5] SA. Igwe, DN. Akunyili, C. Ogbogu. Effects of *Solanum melongena* (garden egg) on some visual functions of visually active Igbos of Nigeria. Journal of Ethnopharmacology, 86, 135-138, 2003.
[6] PR. Guimarães, AMP. Galvão, CM. Batista, GS. Azevedo, RD. Oliveira, RP. Lamounier et al. Eggplant (*Solanum elongena*) infusion has a modest and transitory effect on hypercholesterolemic subjects. Brazilian Journal of Medical and Biological Research, 33(9), 1027-1036, 2000.
[7] R. Blomhoff , MH. Carlsen, LF. Andersen, DR. Jacobs Jr. Health benefits of nuts: potential role of antioxidants. British Journal of Nutrition, 6(Suppl 2); S52-60, 2006.
[8] JH. Kelly Jr, J. Sabate. Nuts and coronary heart disease: an epidemiological perspective. British Journal of Nutrition, 96 Suppl 2:S61-7, 2006.
[9] KT. Lu, RY. Chiou, LG. Chen, MH. Chen,WT. Tsenget al. Neuroprotective effects of resveratrol on cerebral ischemia-induced neuron loss mediated by free radical scavenging and cerebral blood flow elevation. Journal of Agriculture and Food Chemistry, 19; 54(8):3126-31, 2006.
[10] CC. Yeh, SL.You, CJ. Chen, FC. Sung. Peanut consumption and reduced risk of colorectal cancer in women: a prospective study in Taiwan. World Journal of Gastroenterology, 14; 12(2):222-7, 2006.
[11] CJ. Tsai, MF. Leitzmann, FB. Hu, WC. Willett, EL. Giovannucci. Frequent nut consumption and decreased risk of cholecystectomy in women. American Journal of Clinical Nutrition, 80(1):76-81, 2004.
[12] MC. Morris, DA. Evans, JL. Bienias, PA. Scherr, CC. Tangney et al. Dietary niacin and the risk of incident Alzheimer's disease and of cognitive decline. Journal of Neurology, Neurosurgery and Psychiatry, 75(8): 1093-975(8), 2004.
[13] M. Bes-Rastrollo, J. Sabate, E. Gomez-Gracia, A. Alonso, JA. Martinez, MA. Martinez-Gonzalez. Nut consumption and weight gain in a Mediterranean cohort: The SUN study. Journal of Obesity, 15(1):107-16, 2007.
[14] DA. Moreno, N. Ilic, A. Poulev, IR. Raskin. Effects of *Arachis hypogaea* nutshell extract on lipid metabolic enzymes and obesity parameters. Life Sciences, 78(24), 2797-2803, 2006.
[15] MF. Cesta . Normal structure, function, and histology of the spleen. Toxicology and Pathology, 34:455-465, 2006.
[16] EC. Gordon-Smith.. Haematological effect of drug therapy. Clinical Haematology, 9:572, 1980.
[17] Kim Severson. "Told to Eat Its Vegetables, America Orders Fries" article by in The New York Times September 24, 2010, accessed September 25, 2010.
[18] B.Goldberg, A. Stern. .The mechanism of oxidative hemolysis produced by phenylhydrazine. Academic Press, Inc. New York, 1977.
[19] M. Rokushima, K. Omi, A. Araki, Y. Kyokawa, N. Furukawa, et al. A toxicogenomic approach revealed hepatic gene expression changes mechanistically linked to drug-induced hemolytic anemia. Toxicology Science, 95, 474-484, 2007.
[20] R. Masatomo, O. Kazuo, I. Kae, A. Akiko, F. Naoko, et al. Toxicogenomics of Drug-Induced Hemolytic Anemia by Analyzing Gene Expression Profiles in the Spleen. 2007, http://toxisci.oxfordjournals.org. Acessed 3rd January, 2012.
[21] T. Fujitani, Y. Tada, M. Yoneyama. Chlorpropham-induced splenotoxicity and its recovery in rats. Food and Chemical Toxicology, 42, 1469-1477, 2004.
[22] J. Horton, ME. Ogden, S. Williams, D. Coln. The importance of splenic blood flow in clearing pneumococcal organisms. Annals of Surgery, 195,172, 1982.

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