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Potency of mixed diet of garlic, garden egg and groundnut on some biochemical parameters of induced anaemic wistar rats

M. A. Eluwa, T. E. Isamoh, A. O. Akpantah, O. R. Asuquo and T. B. Ekanem

Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar,
Cross River State, Nigeria

ABSTRACT

This study aimed to evaluate the potency of consumption of mixed diet of garlic, garden egg and groundnut on some biochemical parameters. The animals were divided into five groups labelled 1, 2, 3, 4 and 5. Group 1 served as the non-anaemic control and were 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. Blood samples were collected by cardiac puncture for test of some biochemical parameters such as Total proteins, bilirubin and albumin. Total bilirubin was significantly ($p<0.05$) increased in Group 4 rats as compared to the positive and negative controls. Total protein level was significantly ($p<0.05$) reduced as compared to the positive control while Albumin level showed no significantly ($p<0.05$) among the five groups. Thus a mixed diet of garlic, garden egg and groundnut ameliorate the effect of anaemia on biochemical parameters.

Keywords: Anaemia, Garlic, Garden Egg, Groundnut, Phenylhydrazine, Total Protein, Bilirubin, Albumin

INTRODUCTION

Plants or herbs particularly fruits and vegetables are the cheapest and most common store of nutrients such as carbohydrates, protein, vitamin, minerals and essential amino acid [1]. They are also helpful in treating various diseases [2]. Garlic, garden egg and groundnut are some of such plants commonly consumed in Nigeria not only for their nutritional benefits but also therapeutic benefits. Garlic (*Allium sativum*) has been associated with overall lower risk of spontaneous PTD (preterm delivery) [3], related to a decreased risk of prostate cancer [4] and may help protect against ethanol-induced liver injury [5]. Garden egg (*Solanum melongena*) fruit with its nutritive, pharmacological properties, traditional medicinal uses and non-edible uses have been recently reported [6]. It has also been reported to be beneficial in periodontal diseases [7], inhibits inflammation that can lead to atherosclerosis [8] and beneficial to patients suffering from glaucoma and convergence insufficiency [9]. Groundnut commonly referred to peanuts is a widely consumed legume. Study has demonstrated that short-term peanut consumption might improve lipid profiles, the AIP and CHD risk in free-living hypercholesterolaemic men [10]. Other studies have

demonstrated its health benefits including cardio-protective benefits [11, 12], anticancer [13, 14], and reduced risk of Alzheimer[15, 16].

Phenylhydrazine and its hydrochloride derivatives have been extensively used experimentally to study oxidative haemolysis. Previously, these compounds were used for the treatment of Polycythemia vera [17]. As this drug influence certain biochemical and blood factors, this study was carried out to investigate the possible protective effect of consumption of garlic, garden egg and groundnut on certain biochemical parameters after exposure to phenylhydrazine.

MATERIALS AND METHODS

Breeding of animals

Twenty five adult Wistar rats of both sexes weighing between 150-200g were used. They were obtained from the animal house of Department of Physiology, University of Calabar. They were kept in the animal room 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.

Preparation of the diet

Garlic, garden egg and groundnut were bought randomly from Watt Market located 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 using a kitchen Blender and kept in glass containers with plastic cover to keep them airtight.

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 haemoglobometer (Hemocue Hb 201⁺, Ängelholm, Sweden) using blood samples collected by nipping of rat's tail.

Treatment

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 and fed with normal rat chow;

Group 3 - anaemic, fed with 20g (75%ww) of garlic, garden egg and groundnut in the ratio of 1:1:1 with rat chow;

Group 4 - anaemic, fed with 10g of garden egg (50%ww) in the ratio 1:1 with rat chow

Group 5 - anaemic, fed with 10g of groundnut (50%ww) in the ratio 1:1 with rat chow.

Preparation of serum

Rats of all the groups were anaesthetized with chloroform and blood samples were collected from the animals through cardiac puncture into centrifugal tubes and allowed to clot for 30 minutes. The clotted blood samples were centrifuged to separate the cells from the serum. Sera were then aspirated into labeled vials and stored in the freezer at -15°C until ready to use.

Biochemical studies

Serum levels of Bilirubin was determined based by the method described by [18], total proteins assay was conducted by the method described by [19] while serum albumin levels was examined as described by [20].

Statistical analysis

This was done using analysis of variance (ANOVA) and post hoc test. All values is expressed as mean \pm standard error of mean (SEM) and values are statistically significant at $p < 0.05$.

RESULTS

TABLE I: Serum proteins levels among the five experimental groups

GROUPS	BIOCHEMICAL PARAMETERS		
	Total bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
Group 1	0.41±0.14	6.38±0.53	4.76±0.29
Group 2	0.37±0.24	8.47±1.41	4.42±0.31
Group 3	1.11±0.26	5.52±0.28 ^a	4.87±0.11
Group 4	2.27±0.48 ^{*.a.b}	6.19±0.75	4.92±0.18
Group 5	0.93±0.11 ^c	7.56±0.69	4.91±0.18

Values are mean±SEM, n=5.

*p<0.05 vs control; a=p<0.05 vs grp 2; b=p<0.05 vs grp 3; c=p<0.05 vs grp 4

Group 1= negative control, normal rats fed normal rat chow.

Group 2= positive control, anaemic rats fed normal rat chow.

Group 3=anaemic rats fed normal rats chow with combined diet of garlic, garden egg and groundnut.

Group 4=anaemic rats fed normal rat chow with garden egg.

Group 5=anaemic rats fed normal rat chow with groundnut.

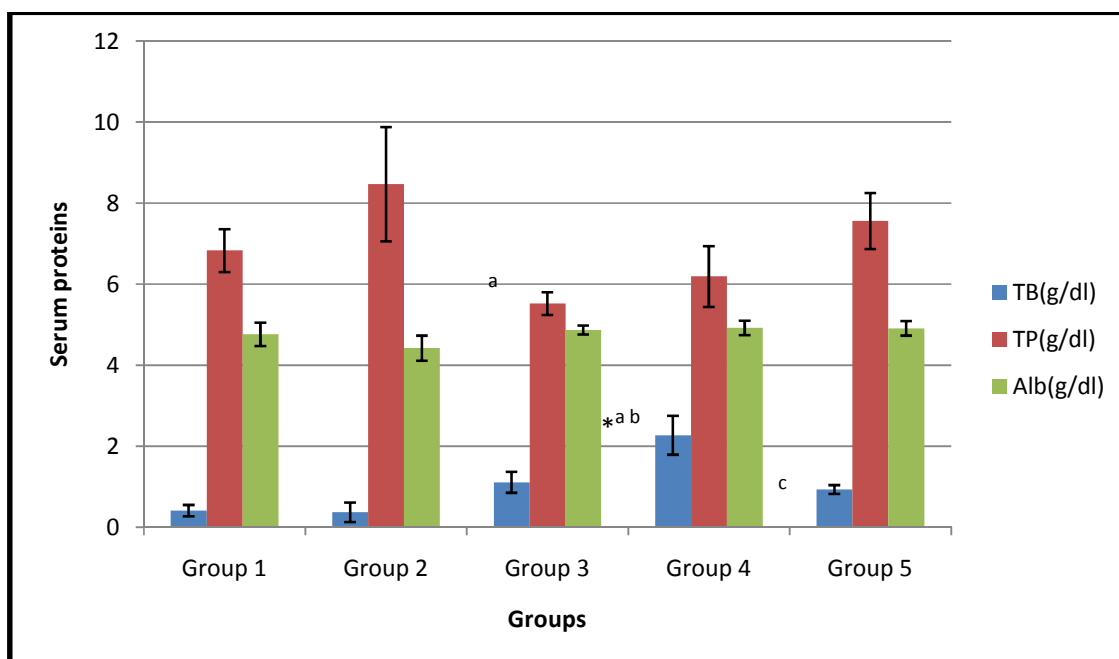


Figure 1: Bar chart showing the serum proteins level among the five experimental groups

Values are mean±SEM, n=5.; *p<0.05 vs control; a=p<0.05 vs grp 2; b=p<0.05 vs grp 3; c=p<0.05 vs grp 4

TB = Total bilirubin; TP = Total protein; Alb = Albumin

In Table I, observed is a slight reduction in total bilirubin in rats in positive control as compared to rats in the negative control. Increase in total bilirubin was observed in anaemic rats fed with the dietary plants, with a significant increase (p<0.05) in rats in group 4 (fed with garden egg) as compared to rats in groups 1, 2 and 3. Total protein level was increase in rats in positive control compared to the negative control. There was a significant decrease in total protein level in rats in group 3 compared to rats in group 2. Groups 4 and 5 animals showed a slight decrease in total protein levels when compared to the animals in group 2. Increased level of albumin is seen in all other groups as compared to the positive control.

DISCUSSION

Evaluation of biochemical indices have been reported to be useful markers in assessing the safety/toxic potentials of botanicals in living cells [21]. The blood chemistry result shows that total bilirubin decrease in anaemic rats fed with normal rat chow as compared to rats in groups 1 and 3. The decrease was significant (p<0.05) when compared to

rats in group 4. Bilirubin is a major breakdown product of old blood cells and increase in bilirubin shows that the liver is functioning well [22]. This effect may be seen as garlic, garden egg and groundnut having a blood enhancing effect.

Total protein was increased in rats in group 2 compared to rats in group 1. The increase was not statistically significant ($p < 0.05$). Total protein levels were decreased in rats in groups 3, 4 and 5 compared to rats in group 2. Factors responsible for the increase in total protein may be excess breakdown of blood protein and increase in tissue protein catabolism.

Albumin levels showed no significant differences among the five groups. It was reduced in the positive control (group 2) than in any other group. Low albumin content in the serum suggests chronic damage to the liver. The reduction in the serum albumin content observed in rats in group 2 may be an indication of diminished synthetic function of the liver. Low serum albumin as obtained in this study may be as a result of malnutrition [23] which occurs during liver damage or infection and or when albumin is lost continuously from the body [24]. In conclusion, consumption of mixed diet of garlic, garden egg and groundnut ameliorate the adverse effect of anaemia on biochemical indices.

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