IMPACT OF NATUAL ANTIOXIDANT ON REDUCTION OF OXIDATIVE STRESS IN HYPERGLYCEMIC RAT FED GERMINATED PIGEON PEA DIET

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INTRODUCTION

- Diabetes affects different people of all ages, race and sex. It is estimated that worldwide there are approximately 150 million people with diabetes mellitus. Diabetes is a metabolic disorder characterized by hyperglycemia and insufficient secretion or action of endogenous insulins.
- Oxidative stress plays a major role in the pathogenesis of diabetes. This increased oxidative stress is accompanied by a decreased antioxidant capacity.

INTRODUCTION (*Cont.*)

- Usage of antioxidants helps in reducing risk of oxidative damages in diabetic patients but synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) may be inappropriate for chronic human consumption, as some publications have mentioned their possible toxic properties for human health(Yang, 2006). There is growing interest towards natural antioxidant from herbal sources

INTRODUCTION (*Cont.*)

- Legume seeds are a rich source of many substances with antioxidant properties, including plant phenolics. Pigeon pea is a potential source of bioactive compounds with antioxidant activities. Germinated pigeon pea has more obvious biological activities and more plentiful secondary metabolities than the ungerminated counterpart. Relevant biosynthetic enzymes are activated during the initial stages of germination.

INTRODUCTION (*Cont.*)

- In this present work both antioxidant, free radical scavenging and α-amylase and α-glucosidase inhibitory potential of germinated pigeon pea and influence of antioxidant activity by measuring the level of blood glucose concentration, lipid peroxidation and non protein sulphydryl group in the liver tissues of diabetes induced high cholesterol fed rats were investigated.

MATERIALS AND METHODS

- Dried red variety of pigeon pea (*cajanus cajan*) were purchased from Ogbete Main Market in Enugu State – Nigeria. The samples were contained in plastic sealed and stored in refrigerator at 4 ^{o}C before germination.

Germination Process

- The pigeon pea was soaked in 250 ml of water containing 0.7% sodium hypochlorite solution for 30 minutes at room temperature. The seeds were drained off, watered to neutral pH, and soaked in distilled water for 5 hours and the hydrated seeds were placed under wet muslin cloth and left to germinate for 3 days at room temperature (28 ^{o}C) without direct contact with sun light . The sprouted seeds were oven dried at 60 ^{o}C for 4 hours and ground to pass 0.18 mm sieve to obtain the flour. The non sprouted seed was ground and sieved. This served as control. Part of the flour samples were defatted and stored in air tight container polythene bag at 0 ^{o}C until it was used.

- Determination of Total Phenolics
- Determination of DPPH free Radical Scavenging Activity
- α-Glucosidase and α-Amylase Inhibition Assay

Animal and Diet

- Adult wistar –albino rats weighting between 150 – 200g purchased from the Department of Animal Science, University of Nigeria Nsukka, Enugu State, Nigeria were used in the experiments. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions, in their individually and partly restricted metabolic cages (16 hours before the experiments, they were fasted overnight, but allowed free access to water). Six rats were used for each group of study.

Induction of Diabetes

- Diabetes mellitus was induced by a single interperitonial injection of ice cold alloxan monohydrate freshly dissolved in normal saline (2%) at a dose of 180mg/kg body weight. Single intraperitonial injection of normal saline was given to animal in the control group. After 7 days, the fasting blood glucose (FBG) level of test animal was measured and only rat with FBG level more than 220mg/dl were used for the study.

Experimental Design

Eighteen rats were divided into three groups, each consisting of six rats.

- Group 1: Normal control, rats were non-diabetes induced and were given normal diet.
- Group 2: Rats were diabetes induced fed with high cholesterol diet.
- Group 3: Rats were diabetes induced fed with high cholesterol diet plus germinated pigeon extract.

After four weeks of treatment, the blood was collected and the animal decapitated. The study was performed in accordance with the International Guidelines regarding animal experiment .

- Determination of Blood Glucose Levels
- Determination of Lipid Peroxidation (LPO) on Liver
- Estimation of Non-Protein Sulphydryl Group (Cellular GSH) in Liver

Statistical Analysis

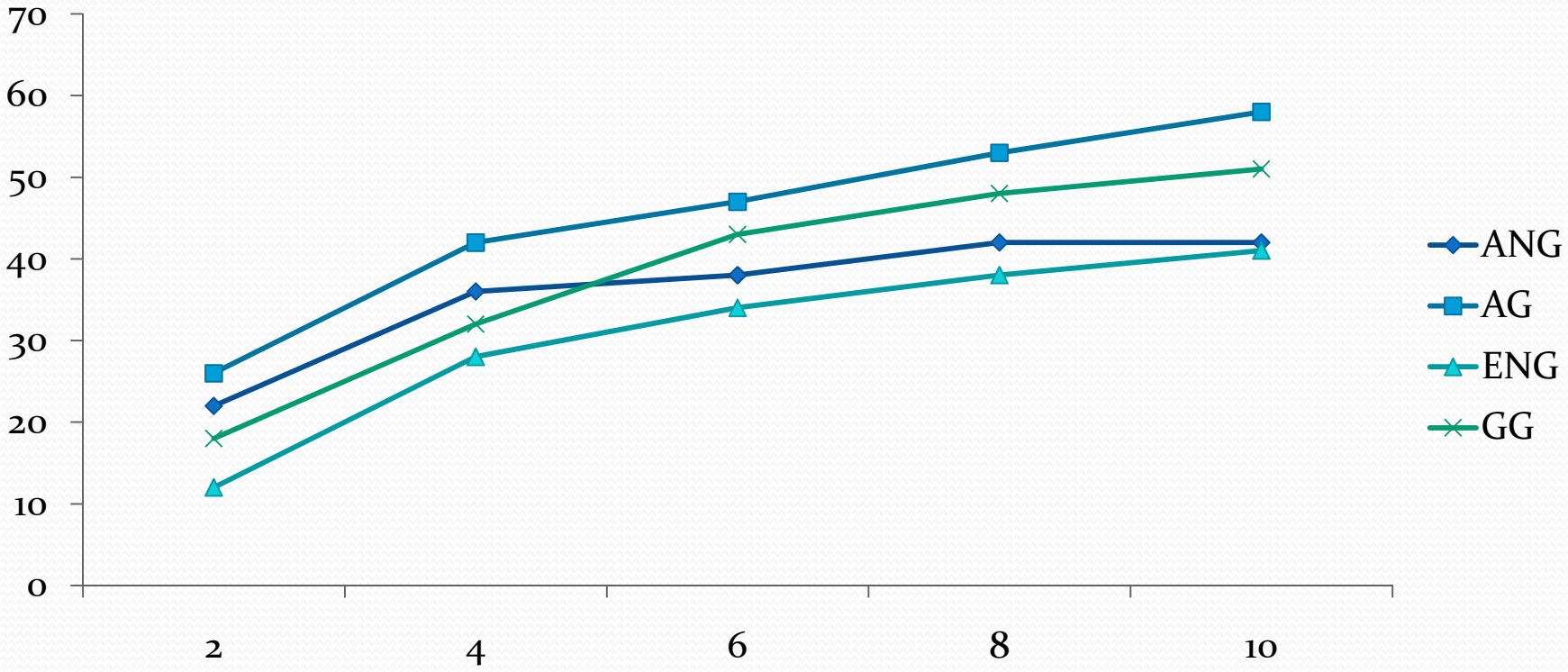
- Data was subjected to analysis of variance using the statistical package for social science (SPSS), version 15.0. Results were presented as mean ± standard deviations. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $p<0.05$ using the Duncan Multiple Range Test values are average of triplicate experiments ± standard deviation.

RESULTS

Table 1: Total Phenolic content (TPC) and DPPH in germinated and non-germinated pigeon pea extract

Assay	Sample	Mean ± SD
TPC (mg GAE/100g dry weight)	Germinated pigeon pea	95.01 ± 0.02[a]
	Non germinated pigeon pea	73.02 ± 0.002[b]
DPPH (µm/ml)	Germinated pigeon pea	85.2 ± 0.02[a]
	Non germinated pigeon pea	52.1 ± 0.04 [b]

Fig 1: α-amylase and α-glucosidase inhibitory potential of germinated and non germinated pigeon pea seed.



AG - α-amylase inhibitory potential of germinated pigeon pea seed.
ANG - α-amylase inhibitory potential of non germinated pigeon pea seed.
GG - α-glucosidase inhibitory potential of germinated pigeon pea seed.
GNG - α-glucosidase inhibitory potential of non germinated pigeon pea seed.
Each value represents mean ± SD, and the significance accepted at $p<0.05$.

Fig. 2: Effect of germinated pigeon pea diet on blood glucose levels in normal and alloxan – induced diabetic wistar rats for 4 weeks.

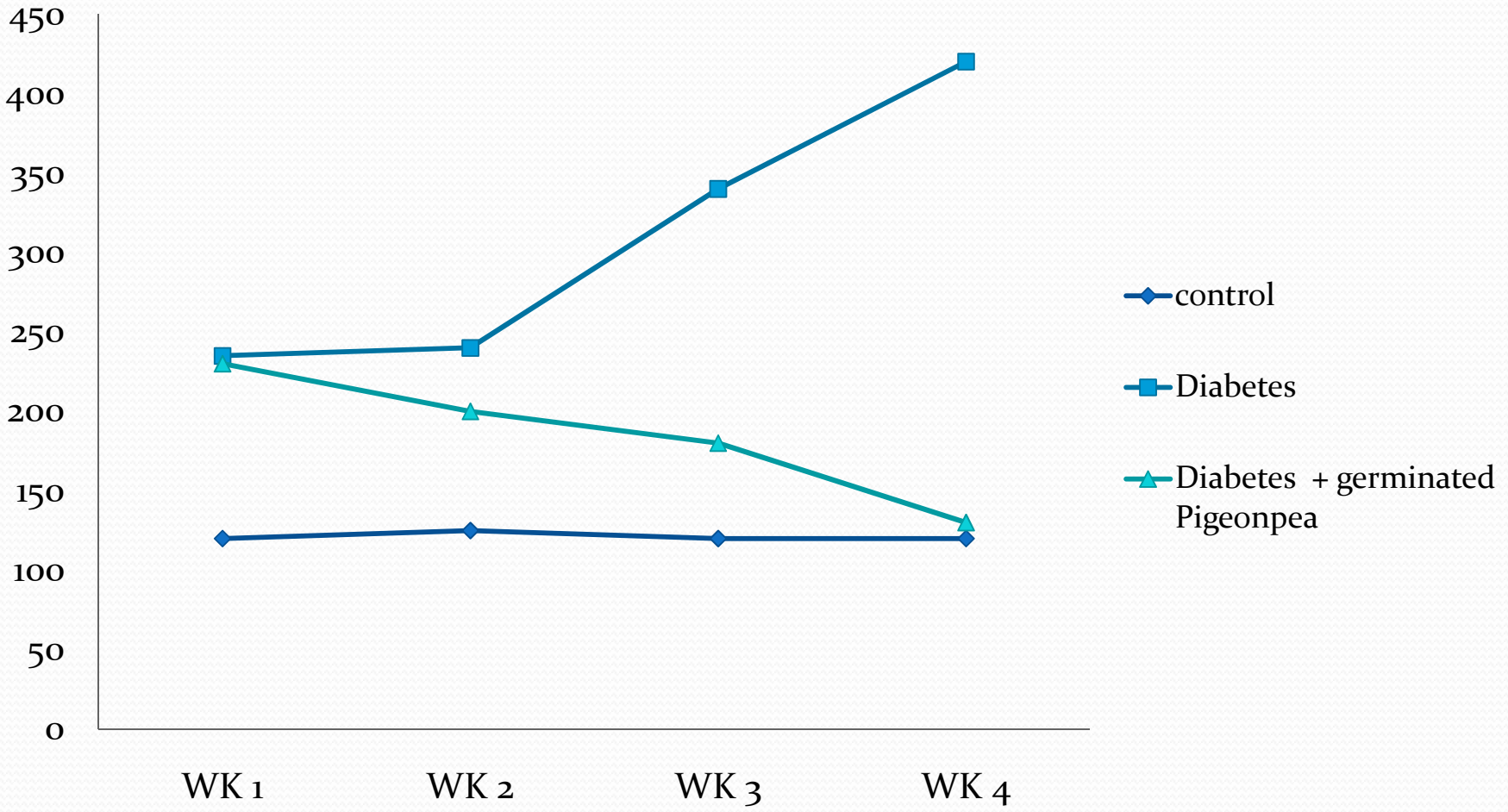


Table 2: Effect of oxidative stress markers in control, hyperlipidemic and germinated pigeon pea extract treated diabetic rats.

Groups	Control	Alloxan + cholesterol	Germinated pigeon pea extract 2.1g/kg bw
Lipid peroxidation n mol MDA release/mg protein	1.31 ± 0.22	2.01 ± 0.25[a]	1.43 ± 0.33[b]
GSH µg reduced GSH utilized /mm/mg protein	30.2 ± 1.7	15.4 ± 2.1[a]	28.3 ± 1.7[b]

Values are mean ± SD
MDA – Malondialdehyde, GSH – Reduced glutathione.

CONCLUSION

- The free radical scavenging activity of germinated pigeon pea extract in diabetes – associated hyperlipidemia has been demonstrated in this study. This may be related to the high amount of total phenolics, increased antioxidant potential and inhibitory potential of carbohydrate-digesting enzymes. The present study concluded that consumption of germinated pigeon pea can be good dietary supplement for controlling diabetes and hyperlipidemia.

THANKS FOR LISTENING !!!