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Effects of Unprocessed Pigeon pea (*Cajanus cajan*) on the Reproductive Antioxidant Indices of Male Wistar Rats

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Abstract

The present study investigated the influence of unprocessed pigeon pea (*Cajanus cajan*) on the antioxidant profile in the testes of rats. Thirty male Wistar rats were assigned into six groups (A-F) of 5 rats each. Group A was fed 10% pigeon pea inclusion diet, group B: 20% pigeon pea inclusion diet, Group C: 30% pigeon pea inclusion diet, Group D: 40% pigeon pea inclusion diet, Group E: 100% pigeon pea diet, while Group F rats served as the control and they were fed with commercial rat concentrates feed. All the rats were given 30g of feeds per day for 21 days with water *ad libitum*. All analysis was done using standard methods. The results showed that malondialdehyde (MDA) concentration was significantly ($p < 0.05$) decreased in all the feed inclusion groups. The reduced glutathione (GSH) concentration was however increased significantly ($p < 0.05$) in the 20% and 40% inclusion groups compared to the control. The catalase activity in the testicular tissues also decreased significantly ($p < 0.05$) below the control group across board. The superoxide dismutase (SOD) activity was significantly ($p < 0.05$) increased using the control as a reference point in both the 10% and 30% treatment groups. There was a significant increase in the total protein concentration in the 10% and 40% treatment group while the 20% feed inclusion showed a significant ($p < 0.05$) decrease. The concentrations of H_2O_2, was significantly decreased in all the treatment groups. In this study, it was discovered that the unprocessed pigeon pea (*Cajanus cajan*) seeds did not cause oxidative stress on the reproductive status of the male rats as shown by the low levels of hydrogen peroxide and MDA and an appreciable increase in the activities of endogenous antioxidants enzymes namely SOD and GSH.

Keywords: *Pigeon Pea; Testes; Antioxidants; Rats*

Introduction

Pigeon pea (*Cajanus cajan)* (L.) Millsp., also known as red gram is a popular and multipurpose grain legume crop, which is commonly found and consumed in semi-tropical and tropical developing countries [1]. Pigeon pea is an important legume crop in Nigeria, used as both a food crop and a cover crop and it is a good source of protein to animals. It is widely cultivated in Nigeria, especially the middle-belt and the Northern region [2].

In comparison with other grain legumes, pigeon pea is the sixth in area and production, but it is utilized in more diverse ways than all the other grain legumes [3-6].

Antioxidants are substances that suppress free radical-mediated oxidation through the inhibition of formation of free radicals by scavenging radicals [7] and radical scavenging action depends on the concentration and reactivity of the antioxidant [8]. Antioxidants are widely used as food additives to act against oxidative stress caused by free radicals [9].

Reactive oxygen species (ROS) are continuously generated during the normal processes of cellular metabolism and also by environmental factors like radiation, pollution, smoking, toxins and toxic chemicals [1]. An imbalance between ROS production and the body's antioxidant defense system results in an accumulation of ROS, which induces cell damage by modifying molecules, including proteins,

lipids, and DNA [10], leading to oxidative stress that is associated with tissue damage, accelerated aging, and degenerative changes.

Despite the numerous studies that have been carried out on *Cajanus cajan*, an important grain legume, there is paucity of information in literature on the male reproductive antioxidant status of unprocessed pigeon pea seeds, since animals feed on unprocessed plant materials under natural conditions.

Aim of the Study

The aim of the present study is to evaluate the effects of unprocessed *Cajanus cajan* on the reproductive antioxidant indices of male Wistar rats.

Materials and Methods

Experimental Protocols

Thirty male Wistar strain rats weighing between 100g and 120g, were bought from the experimental animal house of the Department of Physiology, University of Ibadan, Ibadan, Nigeria. The animals were kept in stainless-steel individual metabolic cages (Associated Crate Ltd), at the Department of Animal Science, University of Ibadan, Nigeria. They were allowed to acclimatize for a period of two weeks. The rats were assigned into six groups (A-F) of 5 rats each. Group A was fed 10% pigeon pea inclusion diet, group B: .20% pigeon pea inclusion diet, Group C: 30% pigeon pea inclusion diet, Group D: 40% pigeon pea inclusion diet, Group E: 100% pigeon pea diet, while Group F rats served as control and they were fed with commercial rat concentrates feed. All the rats were given 30g of feeds daily for 21 days with water *ad libitum*. All the rats were then sacrificed, and the right testes harvested according to standard protocols. Institutional ethical approval was obtained before the start of the research.

Feed Preparation

The *C. cajan* seeds and the rat concentrate feed (3 kg each) were ground into powdery form using an electric miller. The feed was then reconstituted into different percentage inclusions of pigeon pea seed (10%, 20%, 30%, 40%, 100%) and normal concentrate feed as control [11].

Chemicals

Epinephrine, glutathione (GSH), thiobarbituric acid, 5, 5'-di-thiobis-2-nitrobenzoic acid, hydrogen peroxide and 1-chloro-2, 4-dinitrobenzene (CDNB) were bought from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Biochemical Assays for testes antioxidant enzymes

All the right testes were homogenised in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15 % potassium chloride and the homogenate was centrifuged at 10000g for 15 minutes at 4°C and the supernatant was collected for biochemical assays. Reduced glutathione (GSH) level was determined at a wavelength of 412 nm according to the method of Jollow., et al. [12]. Hydrogen peroxide (H_2O_2) generation was determined using the method of Wolff [13]. Lipid peroxidation, quantified as malondialdehyde (MDA) was determined according to the method of Farombi., *et al*. [14] and results were expressed as micromoles of MDA per milligram of protein. Superoxide dismutase (SOD) activity was assayed with the method of Misra and Fridovich [15]. Catalase (CAT) activity was assayed using hydrogen peroxide as substrate using the method of Clairborne [16]. Protein concentration was determined by the method of Lowry., *et al* [17].

Results

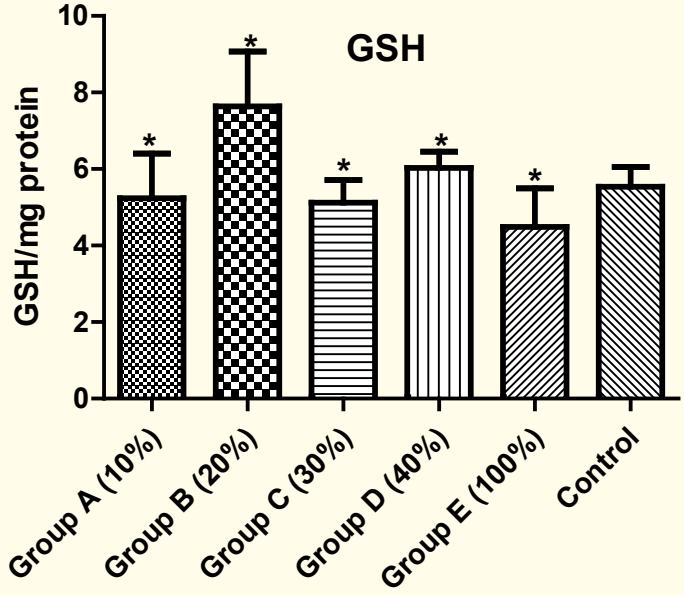

Figure 1: *Levels of GSH in the testes of rats after consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.*

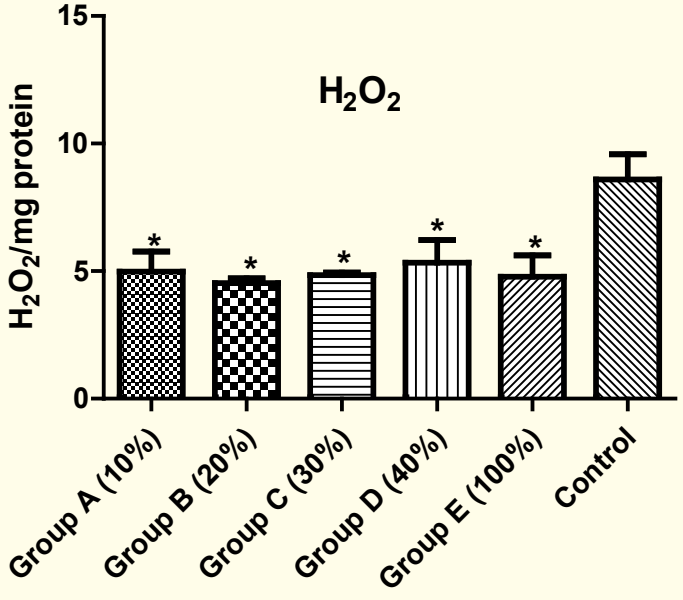

Figure 2: *Levels of H_2O_2 in the testes of rats after consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.*

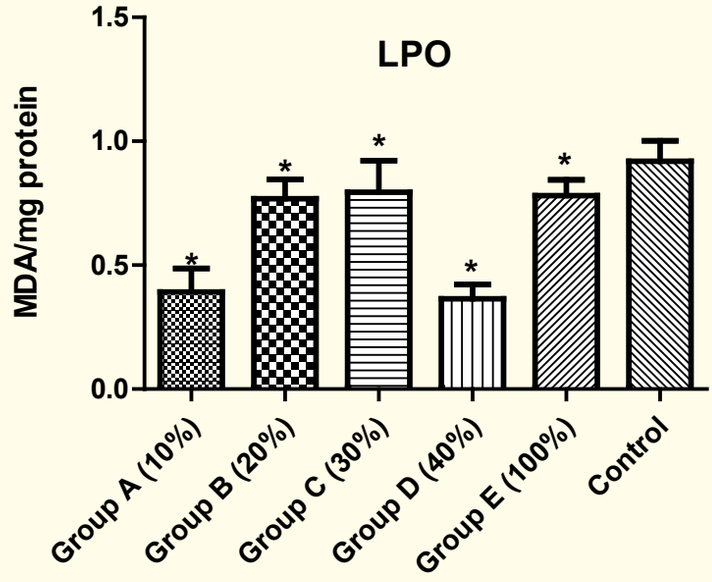

Figure 3: *Levels of LPO (quantified as MDA) in the testes of rats after consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.*

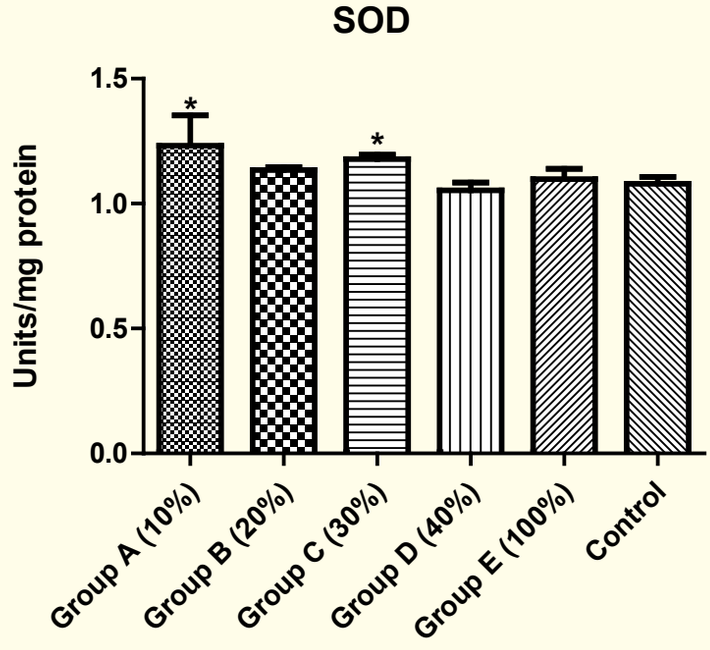

Figure 4: *Activities of SOD in the testes of rats after consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.*

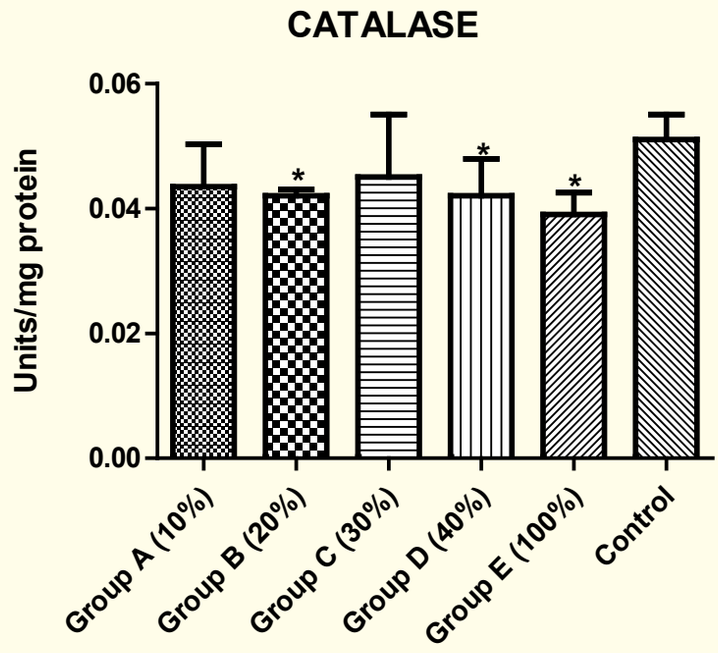

Figure 5: *Activities of catalase in the testes of rats after consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.*

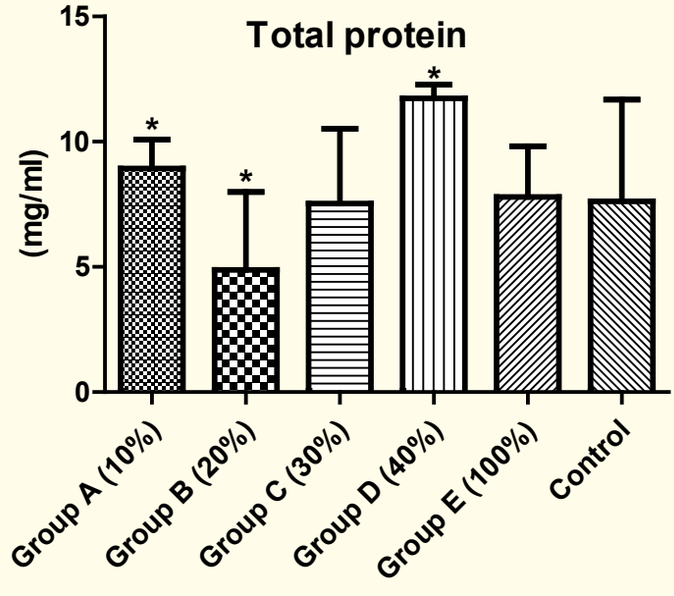

Figure 6: *Total protein concentration in the testes of rats after consumption of unprocessed pigeon pea for 21 days. Values are expressed as mean ± S.D of five rats. *P < 0.05 versus Control.*

Discussion

The present study revealed that the GSH level increased significantly ($p < 0.05$) in the 20% and 40% inclusion groups compared to the control. GSH has been reported to play an important role in protecting tissues from oxidative damage. GSH reduces H_2O_2 via a reaction catalyzed by glutathione peroxidase [18]. It is also a scavenger of singlet oxygen and hydroxyl radical. GSH-dependent enzymes also serve in detoxification metabolism especially against hydrogen peroxide [19]. Accumulation of H_2O_2 degrades methemoglobin, producing ions that reacts to form a species that appears to be OH. Oxyhemoglobin reacts with low concentrations of H_2O_2 to form reactive species that degrades deoxyribose [20].

MDA level is an indicator of lipid peroxidation and the production of MDA is used as an indicator of oxidative stress biologically [21]. MDA production arises from the generation of a hydroxyl radical in the Fenton reaction. Hydrogen atom is extracted, preferably from the double bond of a polyunsaturated fatty acid in a membrane lipid. The chain reaction is continued when O_2 adds to form lipid peroxyl radicals and lipid peroxides. Lipid degradation eventually occurs, forming products like malondialdehyde (from fatty acids with three or more double bonds), ethane and pentane (from the ù-terminal carbons of 3 and 6 fatty acids respectively), that is assayed in the tissues or plasma [22].

The concentrations of H_2O_2, was significantly decreased in all the treatment groups. MDA level was significantly ($p < 0.05$) decreased in all the feed inclusion groups. The SOD activity was significantly ($p < 0.05$) increased using the control as a reference point in both the 10% and 30% treatment groups. The catalase activity in the testicular tissues also decreased significantly ($p < 0.05$) below the control group across board. There was a significant increase in the total protein concentration in the 10% and 40% treatment group while the 20% feed inclusion showed a significant ($p < 0.05$) decrease.

Superoxide dismutase, which is the first line of defense system against oxygen-derived radicals is responsible for the dismutation of superoxide radicals to H_2O_2, while catalase metabolically removes the H_2O_2 from the intracellular environment, leading to the further reduction of the H_2O_2 and hydroxyl radical generation [23]. Superoxide dismutase is present in high concentrations in all tissues and it has a high catalytic efficiency, hence, it provides the cells with a high degree of cellular protection against superoxide anion under normal condition. Superoxide dismutase is localized in the nuclei, and in the seminiferous tubules of the testes [24].

Catalase is a tetrameric enzyme containing four heme groups that allow the enzyme to react with hydrogen peroxide and it functions to catalyze the breakdown of hydrogen peroxide to water and oxygen [25,26]. An increase in SOD assayed from the 10% and 30% treatment groups and a sufficiently high levels in all other feed inclusion groups could suggest that no oxidative stress on the reproductive status of male rats.

Conclusion

This study concluded that the unprocessed seeds of pigeon pea (*Cajanus cajan*) did not cause oxidative stress on the reproductive capacity of the male rats, as shown by the low levels of hydrogen peroxide and MDA and an increase in endogenous antioxidant status in the testes of unprocessed pigeon pea-fed rats.

Conflict of Interest

There is no conflict of interest in this study.

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